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On the Branchial Vessels of Sternaspis.

By

Edwin S. Goodrich, M.A.,
Fellow of Merton College, Oxford.

With Plates 1 and 2.

Some years ago, when studying the interesting worm Sternaspis thalassemoides, Otto, at the Zoological Station at Naples, for the purpose of describing the structure of its excretory and reproductive organs (**2**), I examined the very remarkable and beautiful vascular apparatus which supplies the gill filaments at the hind end of the body. Finding that the branchial organs of Sternaspis did not appear to agree in the details of their organisation with any of the descriptions hitherto given, I determined to work out their minute anatomy. But owing to their very small size, to the presence of a tough cuticle, and to an external layer of sandy particles, it is very difficult indeed to make out the exact relation of the various blood-vessels to the gill filaments, either by dissection or by serial sections. It is, therefore, only after repeated failures, that it is at last possible for me to present what is, I believe, a correct account of their structure.

Max Müller mentioned the dorsal branchial vessels of Sternaspis in 1852, and some years later Claparède figured them and briefly described them. Each blood-vessel, according to Claparède, is "accolé à un axe solide, élastique et cylindrique . . . . . de consistance cartilagineuse," which is said to be surrounded by a "série d'anneaux musculaires" (**2**).

The first detailed account of the blood-supply of the gills is given in Vejdovsky's great memoir (**5**). He describes two bundles of "branchial arteries" springing from the dorsal

vessel, and running to the perforated plates on either side of the anus, through which they reach the gill filaments. The "artery" passes up the filament to the tip, where it turns round to return to the base, and issues as a minute ventral "vein." These veins are collected together on each side into a large lateral branch of the median ventral vessel running above the nerve-cord. The dorsal "arteries" are distinguished by the possession of a peculiar "axis," formed of an outer sheath of ring-shaped cells with regularly arranged nuclei, surrounding an internal "knorpelartiger elastischer Strang welcher . . . . . aus den Zellen zusammengesetzt erscheint." The cells of this inner strand are said to correspond to those of the outer sheath, and to have a row of nuclei. Both blood-vessel and axis are described as surrounded by a common sheath of peritoneal epithelium. The dorsal vessel is supposed to pump the blood forwards, the circulation being from the veins to the branchial filaments, and from these through the arteries to the dorsal vessel. The gill filament itself Vejdovsky describes as having an outer layer of epidermis, below which are muscles; a median longitudinal septum runs down the filament separating two cavities, lined by epithelium, in which are the artery and vein.

Shortly after the appearance of this work Rietsch published an elaborate account of the vascular system of Sternaspis (**4**). I have been able to confirm most of his excellent description. Curiously enough neither this author nor Vejdovsky seem to mention the interesting horizontal septum, formed of a double layer of cœlomic epithelium pierced here and there with holes (fig. 1, *hs*), which stretches across the posterior region of the cœlom from the genital ducts to the rectum. This septum supports the lateral segmental branches of the ventral vessel, and incompletely separates the body-cavity into an upper chamber containing the intestine and gonads, and a ventral chamber in which project the inner ends of the chætæ placed round the ventral shield, and the nerve-cord.

Rietsch's account of the branchial apparatus is less satisfactory than that of Vejdovsky. According to the former,

the branchial vessels "se composent d'un axe conjonctif et d'un vaisseau paralléles et enveloppés dans une gaine commune." Further, "l'axe se compose d'une serie d'anneaux enveloppant un cylindre fibreux. Le dernier est constitué par des fibres longitudinales munies de noyaux allongés" (**4**). On the whole Rietsch's interpretation of the structure of these vessels is very much the same as Vejdovsky's; but he believes the "axis" to be continuous behind with the epidermis, of which it is considered to be a prolongation. He is not clear as to the exact relation of the dorsal and ventral branchial vessels to the filaments. Rietsch, indeed, is not certain that the ventral vessels enter the gills at all, and believes that they may only supply the body-wall, pointing out that they are fewer in number than the dorsal "arteries." He denies the contractility of the main dorsal vessel, and suggests that the blood may be propelled by the lengthening and shortening of the axis supporting the "arteries." The gill filament is said by him to contain only one vessel, and the cavity not to be lined by peritoneum.

In answer to Rietsch, who criticised his work, Vejdovsky published a second more detailed, but scarcely more correct, account of these complicated organs (**6**). Here the branchial "veins" are accurately described and figured; the "axis" of the branchial "arteries" is said to consist "aus einer hyalinen, bindegewebigen Substanz . . . . an dessen Wandung in zierlicher Anordnung vielfach verästelte Zellen gelagert sind," surrounded by contractile "Halbringen" covered with an outer hyaline sheath of cells with large nuclei situated in a row.

As already mentioned, according to my own observations, the structure of the branchial apparatus differs considerably from that described by these authors.

The slender outer gill filaments, as is well known, are capable of independent movement, and may be quickly retracted into a closely coiled spiral (see **4**, **5**, and **3**, fig. 16; also Pl. 1, fig. 8). Two small blood-vessels run along each filament, and join at the extreme tip (fig. 2). These vessels

have contractile muscular walls (fig. 4). When the filament is fully expanded the vessels are swollen with blood, and in optical section appear to fill almost the entire cavity of the gill, being separated from each other by a narrow longitudinal septum (figs. 2 and 3, *s*). At other times the vessels may become emptied; their walls then contract, so that the lumen is almost or entirely obliterated. This is the case, as a rule, in preserved specimens; and such gill filaments, when cut in cross-section, present the appearance described by Vejdovsky and Rietsch, of possessing two large cœlomic cavities separated by a strong longitudinal septum. It will be understood, however, that this apparent septum is formed by the collapsed walls of the blood-vessels, and is therefore at right angles to the true septum separating the vessels in a distended condition. Fig. 4 shows these vessels in a half-contracted state. As for the lining of the cavities on either side, it appears to be continuous with the cœlomic epithelium of the body-cavity, although the cells are often very irregularly disposed.

Now, when we come to examine the blood-vessels supplied to the base of the gills, we find that there are not two, but three running to each filament. The main dorsal vessel situated on the intestine (fig. 1, *dv*) gives off behind a short thick branch, which soon divides into two limbs. From the right and the left limb come off in regular alternate succession two rows of offshoots, the dorsal branchial vessels (figs. 1, 7, 8, and 14). These generally expand into two marked swellings, then narrow down to straight vessels running to the branchial perforated plates. It is this region of the branchial "artery" which is said to be supported by an "axis," and it is just this region which has been strangely misunderstood by previous observers.

For the sake of clearness in description we may subdivide the dorsal branchial vessel into three regions: the first is generally marked off as a conspicuous swelling, it is the portion nearest the dorsal vessel; the third is the much longer and narrower region supported by the "axis," and reaching

to the branchial plate, from which the gills arise; and the second region is the intermediate part, generally swollen, and differing in structure from the other two.

Taking the third region first (figs. 1, 12, 13 and 14), we find that it contains a slender blood-vessel with thin walls (figs. 12, 13 and 14, *cv*). This is the branchial artery of Vejdovsky and Rietsch, which we may call the communicating vessel, for reasons which will appear later. Its walls are formed, like those of any other small blood-vessel, of a single layer of granular cells with ordinary rounded nuclei irregularly distributed. The communicating vessel is capable of considerable distension; but in section it generally appears much folded, and with a very contracted lumen (fig. 5, *cv*).

The so-called "axis," along one side of which this vessel is closely applied, is in reality a second blood-vessel with specialised contractile walls. It is in fact the most important blood-vessel in the whole branchial circulation. This highly contractile vessel, which may be called the dorsal branchial vessel, has its walls formed of a regular series of ring-shaped cells, with their large oval nuclei situated in a row on the surface opposite to that to which the communicating vessel is attached (figs. 5 and 13, *n*). These nuclei have been well figured by Vejdovsky (**6**). Inside the dorsal branchial vessel runs a peculiar rod of tissue, to which alone the name "axis" should be applied.

This axial rod consists not of longitudinal fibres, as described by Rietsch, but rather of cartilage-like cells, as mentioned by Vejdovsky in his first memoir (**5**). As will be understood on comparing figs. 12 and 13, it is formed of a slightly irregular row of cells, with a thick hyaline common wall turned towards the cavity of the blood-vessel (fig. 13, *oa*). The cells are attached to the wall of the vessel, on the same side as the communicating vessel lying outside, by means of obliquely placed stalk-like bases. In the living tissue the cells of the axis are seen to present a peculiar vacuolated appearance, with a few highly refractive granules (fig. 12). Lying on the surface of the axis are occasionally seen small

branching cells, which do not appear to form an essential part of the rod, but rather to be amœboid blood-cells creeping over it, such as are found elsewhere in the blood-vessels (figs. 12 and 16). I can find no common peritoneal sheath enclosing the dorsal branchial and the communicating vessels.

The dorsal branchial vessel is capable of undergoing great expansion and contraction. The ring-cells of which it is formed consist of an outer more protoplasmic coat and an inner lining of homogeneous refractive substance. When the vessel is expanded the inner coat appears quite thin; on the contrary, as the lumen contracts the lining becomes correspondingly thickened and folded. In transverse section it then acquires a striated appearance, and is seen to be interrupted along the line where the axis is attached (figs. 5 and 10, *ci*). The thick, contracted, inner lining forms the "Halbringen" of Vejdovsky, and the "bague chitineuse" of Rietsch. It is difficult to determine whether during contraction the function of the inner lining is purely passive. The real agency by means of which the powerful contraction is brought about seems to reside in the superficial network of protoplasmic threads in the outer layer (fig. 6). This remarkable meshwork, which stretches across uninterruptedly in the living tissue from cell to cell, can be seen to undergo changes, the threads becoming slenderer, and the intervening spaces larger as the vessel expands.

Peculiar as the histological structure of the wall of the dorsal branchial vessel appears to be, it may yet be compared to that of the small blood-vessels in Oligochætes so well described by Bergh (1). Here also we have small contractile vessels formed of rows of ring-like cells, the walls of which consist of an inner lining and an outer active protoplasmic network. But in the case of Sternaspis the structure is much more highly specialised.[1]

[1] Since this was written, Lang has published his important work, 'Beiträge zu einer Trophocœltheorie' ('Jen. Zeit.,' 1903). The dorsal branchial vessel appears to correspond in structure to his figs. 10 and 13, pl. 2. The axial rod probably develops as a longitudinal fold and ingrowth of the walls of the vessel.

The contractile dorsal branchial vessel and its contained inner axis form a most efficient apparatus for propelling the blood forcibly from one end of the vessel to the other as waves of contraction pass down it. When fully contracted the lumen is entirely obliterated by the closing of the wall on to the axial rod (figs. 10 and 13).

Passing down to the base of the gill filament we find that the two vessels, the dorsal branchial and the communicating vessel, pass directly into the filament through the pore in the branchial plate, but that the axial rod reaches only to the level of the pore, where it disappears, merging into the septum which separates the two gill vessels.

Following the vessels upwards and forwards towards the intestine, it is seen that at the beginning of what has been termed above the second region the axial rod suddenly diminishes to a thin thread, which runs along the wall of the dorsal branchial vessel and then gradually expands again into a second short axial rod similar to that in the posterior third region (figs. 14 and 15). This short axial piece again thins out to a delicate strand with a nucleus here and there, which is continued forwards into the expanded first region of the vessel attached to its inner surface (figs. 9 and 14). Near the place where the branchial vessel opens by a slightly narrowed neck into the large limb of the dorsal vessel the fine axial strand swells again into a large plug of vacuolated tissue. The plug lies loose in the vessel, kept in place by its posterior attachment, and acts as a valve (figs. 7 and 14).

At the point where the first joins the second region of the branchial vessel the communicating vessel opens into it by an aperture protected by a thin flap acting as a valve, so as to prevent blood passing back into the communicating vessel when the other contracts.

We have seen, then, that two vessels from the dorsal system pass to the base of each gill filament.

Now, the fine ventral branchial vessels, veins of Vejdovsky, also run to the base of the gill filaments. These delicate capillaries pass in near the skin between the dorsal branches,

and may subdivide so that one minute vessel goes to each filament. Since only two vessels are found in each gill filament, and three can be traced to its base, it becomes an interesting matter to determine what becomes of the third. This is the point which I found so difficult to settle.

Whilst it is comparatively easy to follow the dorsal branchial vessel and its accompanying communicating vessel to the base of a gill filament, it is very difficult indeed to trace the course of the ventral capillary vessel. These blood-vessels are too minute to inject or to follow for certain by dissection. Sections taken through the regions where the vessels pass through the branchial plate show that as a matter of fact the communicating vessel joins the ventral branchial vessel quite near the body-wall to form a single vessel entering the gill. Figs. 10 and 11 show this communication clearly, whilst the relation of the three sets of vessels to the gills is represented diagrammatically in fig. 8.

The reason for this peculiar arrangement is not far to seek. Supposing there existed only a dorsal "artery" and a ventral "vein," as described by previous authors, it is obvious that on the retraction of the gill filaments the whole circulation of the blood would be almost entirely stopped. By means of the communicating vessel the blood has in such a case an alternative path open to it leading from the main ventral to the main dorsal vessel. A somewhat similar by-path for the blood is present at the base of the retractile gills of the Urodele amphibians, and serves no doubt the same purpose.

Concerning the circulation of the blood in the living Sternaspis, I feel by no means certain that the direction of the flow is from the ventral vessel to the dorsal vessel through the branchial filaments, as held by Vejdovsky and Rietsch. The disposition of the valves and certain contractions in freshly dissected specimens lead me to believe that the blood is propelled along the contractile dorsal branchial vessels from before backwards. However, this is a subject which requires further study.

List of References.

1. Bergh, R. S.—"Beitr. z. vergl. Histologie," II, 'Anat. Hefte,' vol. xiv, 1900.

2. Claparède.—"Annélides Chetopodes du Golfe de Naple."

3. Goodrich, E. S.—"Notes on the Anatomy of Sternaspis," 'Quart. Journ. Micr. Sci.,' vol. xl, 1897.

4. Rietsch, M.—"Étude sur le Sternaspis scutata," 'Ann. Sci. Nat.,' 6e sér., Zool., vol. xiii, 1882.

5. Vejdovsky, F.—"Unters. über die Anatomie, etc., von Sternaspis," 'Denkschr. d. Wien. Akad. Math.-Naturw. Cl.,' vol. xliii, 1882.

6. Vejdovsky, F.—"Bemerk. z. neueren u. älteren Literatur über Sternaspis scutata," 'Litz. d. k. Böhm. Gesellsch. d. Wissenshaften, 1882.

EXPLANATION OF PLATES 1 & 2,

Illustrating Mr. Edwin S. Goodrich's paper, "On the Branchial Vessels of Sternaspis."

List of Reference Letters.

ax. Axial rod. *axc.* Cell of axial rod. *blv.* Blood-vessel. *brc.* Branching cell resting on axial rod. *c.* Cœlomic canal. *cbw.* Cut body-wall. *ci.* Inner coat. *co.* Outer coat. *cov.* Cut wall of ovisac. *ctt.* Connecting strands of tissue. *cv.* Communicating vessel. *dbv.* Dorsal branchial vessel. *dv.* Main dorsal vessel. *ep.* Epidermis. *gf.* Gill filament. *hs.* Horizontal septum. *i.* Intestine. *ldb.* Limb of dorsal vessel. *ludbv.* Lumen of dorsal branchial vessel. *n.* Nucleus of ring-shaped cell. *na.* Nucleus of axial rod-cell. *nc.* Nerve-cord. *net.* Protoplasmic contractile network. *oa.* Outer hyaline layer of axial rod. *oc.* Outer layer of cuticle. *ovd.* Oviduct. *p.* Point at which the communicating vessel joins the dorsal branchial vessel. *r.* Rectum. *s.* Septum. *sb.* Supporting band of tissue. *stc.* Stalk of the axial rod-cell. *th.* Restraining thread of valvular plug. *vbv.* Ventral branchial vessel. *vf.* Valvular fold. *vp.* Valvular plug. *vv.* Main ventral vessel.

PLATE 1.

Fig. 1.—Enlarged view of a dissection of the hinder region of a female Sternaspis, seen from above. Portions of the ovisac, of the rectum, and of

the intestine have been left, but pushed aside to expose the horizontal septum and ventral vessel.

Fig. 2.—Tip of an expanded branchial filament, enlarged. Fresh.

Fig. 3.—Optical section of an expanded gill filament, enlarged. Fresh.

Fig. 4.—Transverse section of a gill filament in which the blood-vessels are partially contracted. Cam. Z. D, oc. 3.

Fig. 5.—Transverse section of the posterior region of a dorsal branchial vessel, in a semi-contracted condition. Cam. Z. D, oc. 3.

Fig. 6.—Enlarged view of the outer surface of an expanded anterior portion of a dorsal branchial vessel, showing the continuous contractile network. Fresh.

Fig. 7.—Enlarged view of the anterior origin of some of the dorsal branchial vessels. Fresh.

Fig. 8.—Diagrammatic figure of the branchial circulation. One gill filament is expanded and the other contracted.

PLATE 2.

Fig. 9.—Enlarged view of the region where the communicating vessel opens into the dorsal branchial vessel, in optical section. Fresh.

Fig. 10.—Section through two dorsal branchial vessels (contracted) and the accompanying communicating vessels, showing the opening of the latter into the ventral branchial vessels. Cam. $\frac{1}{12}$ oil imm., oc. 3.

Fig. 11.—Section through the same, taken a little farther forward, where the ventral branchial vessels have separated off. Cam. $\frac{1}{12}$ oil imm., oc. 3.

Fig. 12.—Optical section through the dorsal branchial vessel and its axial rod, enlarged. Fresh.

Fig. 13.—Slightly diagrammatic view of the same structures.

Fig. 14.—Enlarged view of the anterior half of three dorsal branchial vessels. Fresh.

Fig. 15.—Enlarged optical section of the region marked with an asterisk in fig. 14.

Fig. 16.—Enlarged view of two amœboid cells in a blood-vessel.

The Middle Ear and Columella of Birds.

By

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It may seem a supererogatory task to add to the pile of literature which deals with the ear-bone homologies a straightforward account of those anatomical and embryological facts which may be ascertained by the examination of such familiar types as the fowl and pigeon; but after a painstaking research into the literature of the Sauropsidan middle ear I have unwillingly concluded that such a course was desirable. Although this literature is voluminous there is no single description of any Sauropsidan type which from a modern standpoint can be considered at all complete; that is to say, there is no account which describes in any one type—

1. The development and transformation of the auditory ossicles from the earliest procartilage stage upwards;

2. The relations of the seventh nerve and chorda tympani to the ossicles at different stages of development.

The words in italics are emphasised because a large part of the work on this subject fails to be conclusive owing to the lack of sufficiently early stages of development, and this most unfortunately is the case in the recent descriptions of Sphenodon by Howes (**14**) and Schauinsland (**12**). Kingsley (**13**) gives one isolated procartilage stage in a Lacertilian;

which serves to prove, at any rate, that these early stages are absolutely necessary for the interpretation of the later.

The following essay will be divided into three parts :—(1) anatomical, in which certain new details are described, and an adequate account of the disposition of the chorda tympani is given for the first time ; (2) embryological, in which special attention is paid to the derivation and homology of the stapes or proximal portion of the columella (an homology which constitutes the crux of the Sauropsidan middle ear) ; and finally (3) a summary with some general conclusions.

I am much indebted to Mr. Jenkinson, Lecturer in Embryology in the University Museum, for his advice and a great deal of material.

1. Anatomy.

The Columella (Fig. 1)—Anatomically the columella of birds is composed of two pieces, an inner ossified piece, the stapes, apposed to the fenestra ovalis, and an outer cartilaginous piece, the extra-columella, united to the stapes proximally, and attached distally to the tympanic membrane. There is no real joint between the stapes and extra-columella, but great flexibility exists between the two, owing to the pliability of the cartilaginous neck which unites them. The extra-columella may be described as consisting of three pieces, supra-, extra-, and infra-stapedial, all perfectly continuous. The disposition of these parts is shown in fig. 1, which represents the left columella of Gallus, viewed from within the tympanum.

The columella is supplied with a single muscle, the tensor tympani, which is attached to the infra-stapedial, and to the edge of the tympanic membrane, between the infra- and extra-stapedial cartilages. The muscle passes out of the ear by a large foramen close to the stylo-mastoid foramen, curls round on to the back of the skull, and is broadly attached to the basi-occipital bone in a shallow groove which slopes nearly to the occipital condyle.

The extra-columella is supplied with one ligament in all birds, Platner's ligament, which stretches across the cavity of the middle ear to the posterior face of the quadrate bone (*Plt.*, Figs. 1 and 3). In Gallus there are present two other ligaments attached to the extra- and infra-stapedials which are in part concentrations of the fibrous constituents of the tympanic membrane; I can only find these erroneously described by Parker (3) as being attached to the quadrate. In reality they pass beneath the quadrate, are continued beyond the region of the tympanic membrane into the lining of the Eustachian tube, and are finally attached to the walls of the bony Eustachian groove near the point where it debouches into the mouth (Fig. 2). This is a peculiar disposition, not found in other birds that I have examined.

The Seventh Nerve.—This nerve has three branches, which are, counting in order from the root of the nerve outwards, the sphenopalatine, the chorda tympani, and the main branch of the seventh. In Gallus the sphenopalatine and the chorda tympani come off together from the geniculate ganglion and do not take up any intimate relation to the middle ear. The chorda tympani, after its origin from the seventh nerve, runs a little way with it in the Fallopian tube, then enters a bony canal of its own and so gains the posterior face of the quadrate. The cross in Fig. 3 shows the approximate point at which the chorda tympani comes off the seventh nerve in the fowl. After giving off the chorda the main branch of the seventh crosses the stapes externally and dorsally to it in the cancellated bone, and then leaves the skull by the stylo-mastoid foramen.

In other birds, e. g. Columba, the chorda has a quite different disposition (Fig. 3). It leaves the seventh nerve by a special foramen in the Fallopian tube just before the seventh nerve makes its exit from the skull by the stylo-mastoid foramen; it then traverses a small piece of cancellated bone and enters the cavity of the middle ear quite superficially, viz. between the extra-columella and the tympanic membrane. It now crosses the extra-columella, keeping this same relation

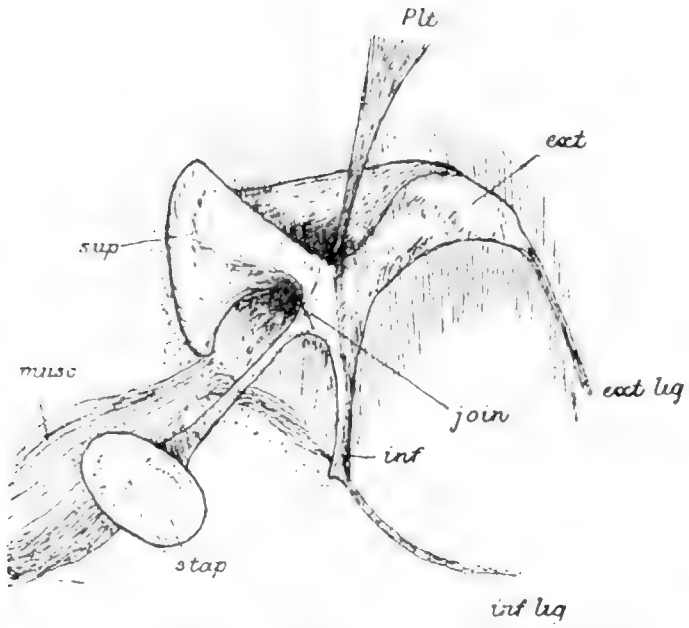


FIG. 1.—Left columella of Gallus from inside tympanic cavity. *plt.* Platner's ligament. *ext.* Extra-stapedial. *ext. lig.* Extra-stapedial ligament. *inf.* Infra-stapedial. *inf. lig.* Infra-stapedial ligament. *sup.* Supra-stapedial. *stap.* Stapes. *musc.* Tensor tympani.

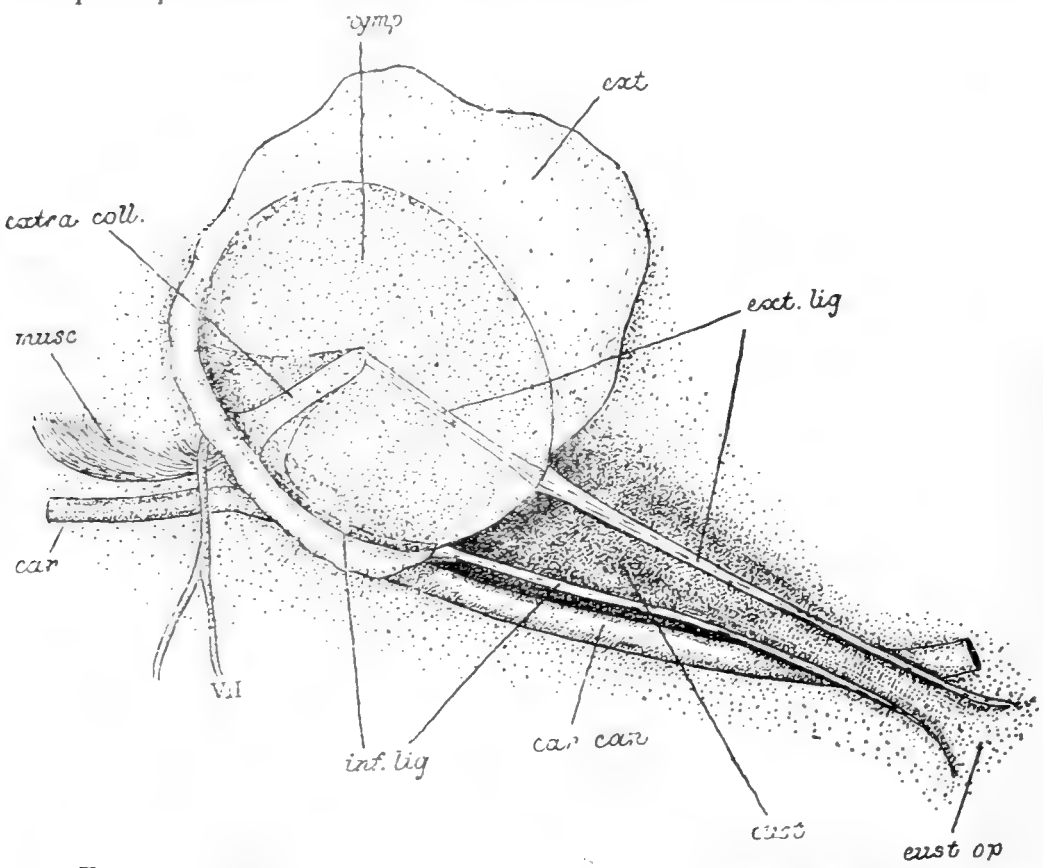


FIG. 2.—Right ear of Gallus. External ear is cut away, and the quadrate and bony roof of the lower tympanic recess are removed. *tymp.* Tympanum. *ext.* External ear lining. *extra coll.* Extra-columellar. *ext. lig.* Extra-stapedial ligament. *inf. lig.* Infra-stapedial ligament. *musc.* Tensor tympani. *car.* Carotid. *car. can.* Bony carotid canal. VII. Seventh nerve. *eust.* Bony Eustachian groove. *eust. op.* Opening of groove into mouth.

to the tympanic membrane, namely lying just internal to it and external to the extra-columella, save that at the point where it crosses the neck which unites the supra- and extra-stapedials it pierces the cartilage superficially.

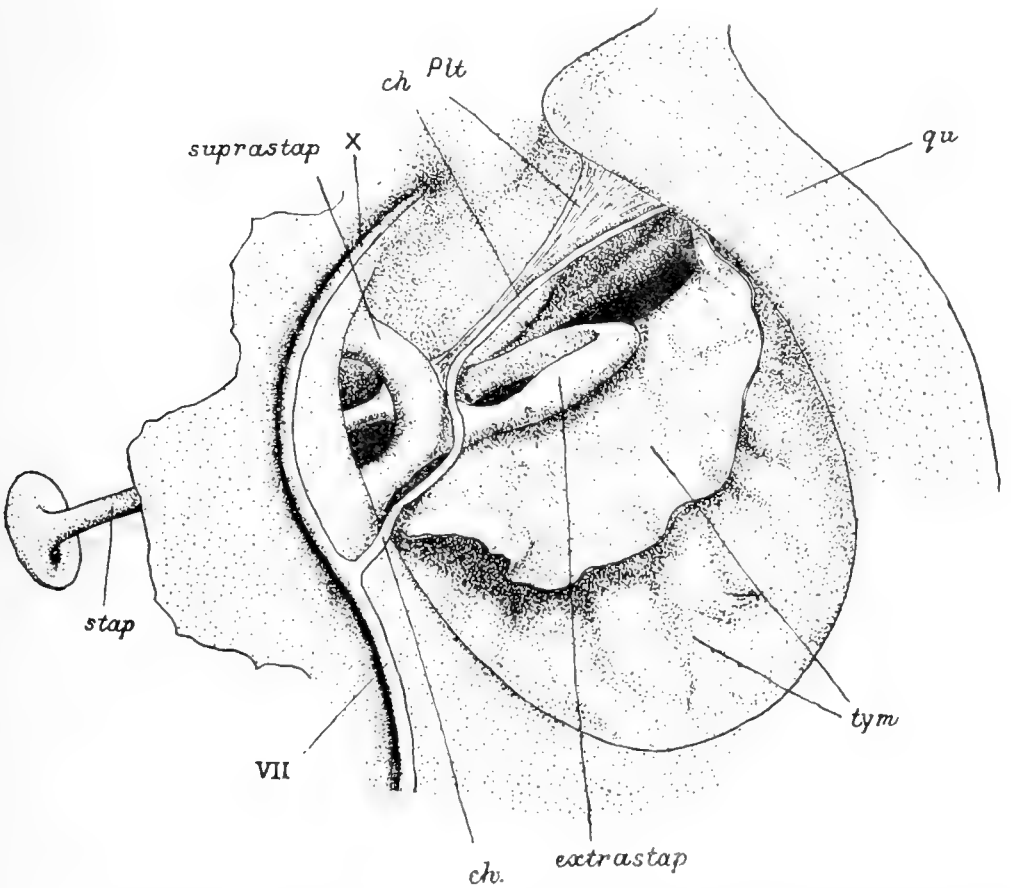
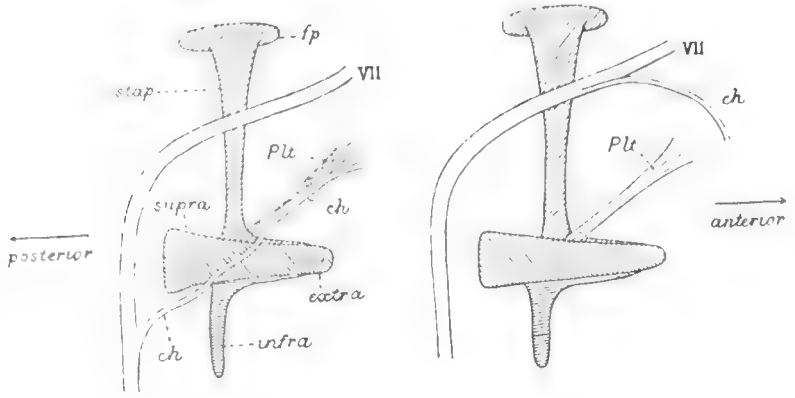


FIG. 3.—Right ear of Columba. Upper half of tympanic membrane deflected to show the structures upon its other side. *stap.* Stapes. *supra stap.* Supra-stapedial. *extra stap.* Extra-stapedial. *plt.* Platner's ligament. VII. Seventh nerve. *ch.* Chorda tympani. × Point at which chorda tympani comes off in Gallus. *tym.* Tympanum. *qu.* Quadrate. For this drawing I am much indebted to Mr. Darbishire.

Having traversed the extra-columella, the chorda joins Platner's ligament and crosses the tympanic cavity in company with it, so gaining the posterior face of the quadrate. This course of the chorda tympani has been confirmed by means of serial sections in a late embryo of the starling.

The essential difference between the relations of the chorda

tympani in Gallus and in Columba may be seen in the following diagram.



I. Columella of Columba; II, of Gallas, from without. *fp.* Foot plug. *stap.* Stapes. *Plt.* Platner's ligament. VII. Seventh nerve. *ch.* Chorda tympani. *supra*, *extra*, and *infra*. Stapedial cartilages.

In these two relations of the chorda tympani to the columella we see a striking convergence towards the two conditions in Lacertilia described by Versluys (**10**). In Lacertilia the chorda tympani may come off the seventh nerve behind the columella, and then run forwards, across, and external or dorsal to the extra-columella, or else it may come off anteriorly to the columella altogether (e. g. Gecko and those forms which have no processus internus to the extra-columella). There can be little doubt that the backward origin is primitive, since Sphenodon shows it, and that the forward origin in the fowl is secondary, as first suggested by Hasse (**2**), who supposed that its forward origin had to do with the peculiar development of the quadrate articulation in that bird.

2. Embryology.

The middle ear cavity is formed from the first gill slit (**5**). The earliest stage which is instructive for the purpose in hand is the five-day-old chick. As yet no chondrification has taken place, but the hyoid arch and the auditory capsule are recognisably shown by the thicker aggregation of connective-

tissue corpuscles in those regions (Fig. 4). The proliferation of tissue to form the hyoid arch takes place from below upwards; this is shown in the figures where the more ventral portion of the arch (*hy.*) is thicker than the more dorsal (*stap.*), the two portions passing into one another more or

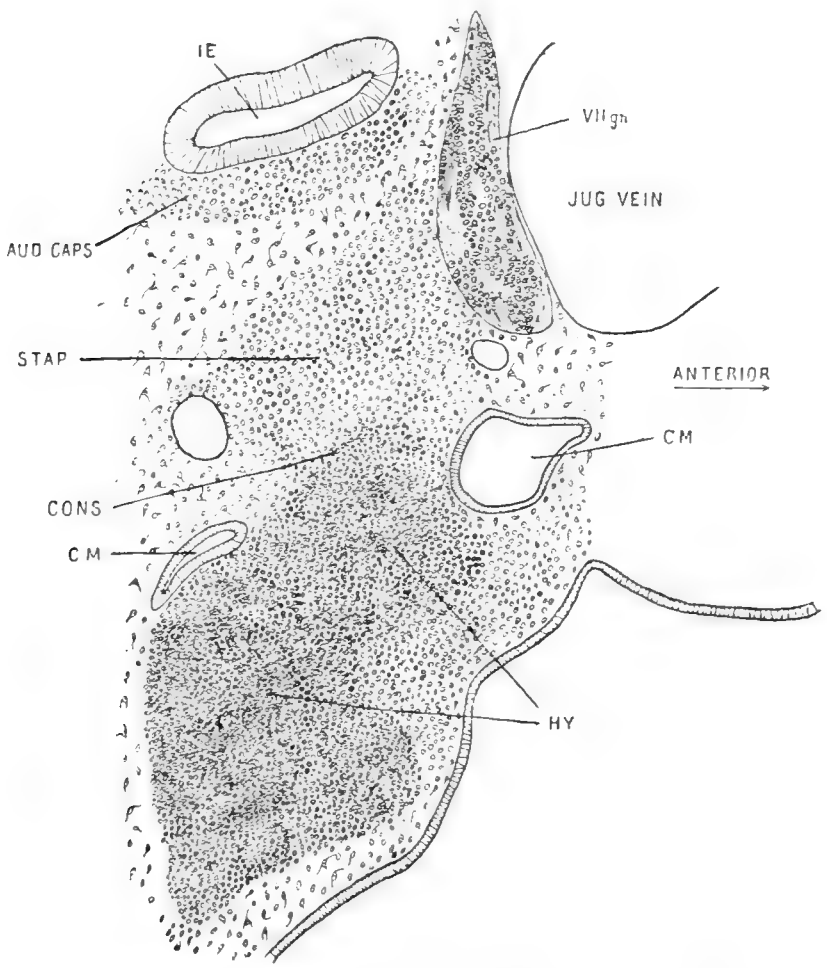


FIG. 4.—Longitudinal (slightly horizontal) section through hyoid region of five-day chick.

less suddenly at the constriction, marked *cons.*, fig. 4. The seventh nerve crosses the hyoid arch just dorsal to the constriction. The hyoid and auditory capsule proliferations are completely separate, being divided by a space where the connective-tissue corpuscles are much more thinly scattered. It is seen in fig. 4 that the dorsal or proximal portion of the

hyoid (*stap.*) has approached quite near to the auditory capsule, while the latter shows no sign of sending an outgrowth to meet it.

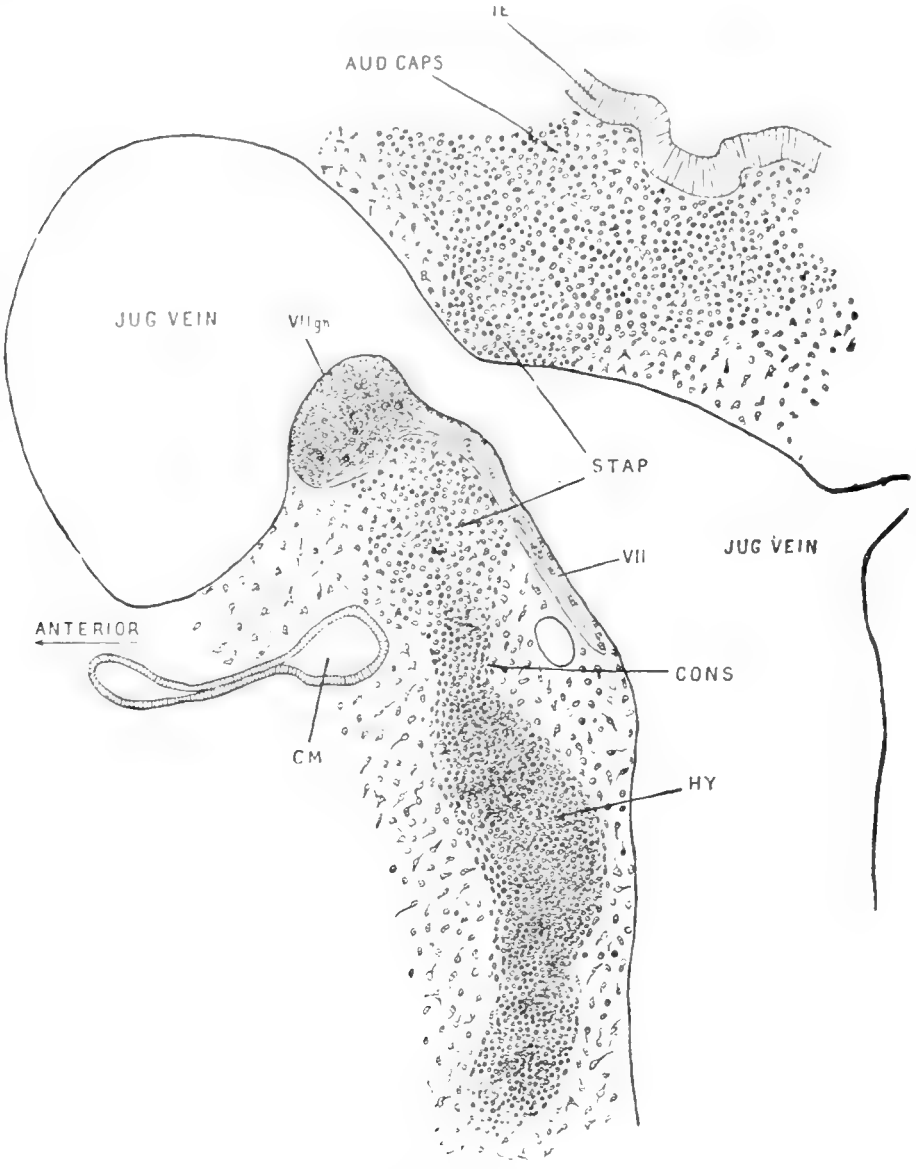


Fig. 5.—Longitudinal section through six-day chick.

In the six-day-chick the top of the hyoid has fused with the auditory capsule, both being still in the pro-cartilaginous condition. This is shown in Figs. 5 and 6. Fig. 5 shows the seventh nerve crossing the hyoid above the constriction in

sensibly the same position as in the five-day-chick. It is quite clear from Figures 4 and 5 that no considerable outgrowth from the auditory capsule can have taken place to complete the continuity of hyoid and auditory capsule. There is no evidence of such an outgrowth, and even if it occurs between the stages Figs. 4 and 5, the outgrowth can only

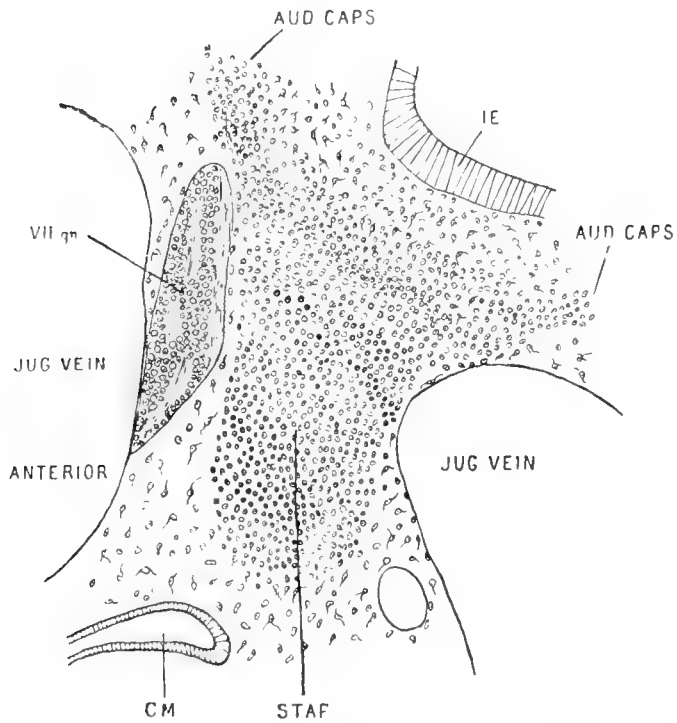


FIG. 6.—Ditto; a more median section to show continuity of stapes with auditory capsule.

Letters used in Figs. 4, 5, and 6:

I. E. Internal ear. AUD. CAPS. Auditory capsule. STAP. Stapes. CONS. Constriction in hyoid arch. HY. Hyoid arch. CM. Cavity of middle ear. JUG. VEIN. Jugular vein. VII gn. Geniculate ganglion. VII. Seventh nerve.

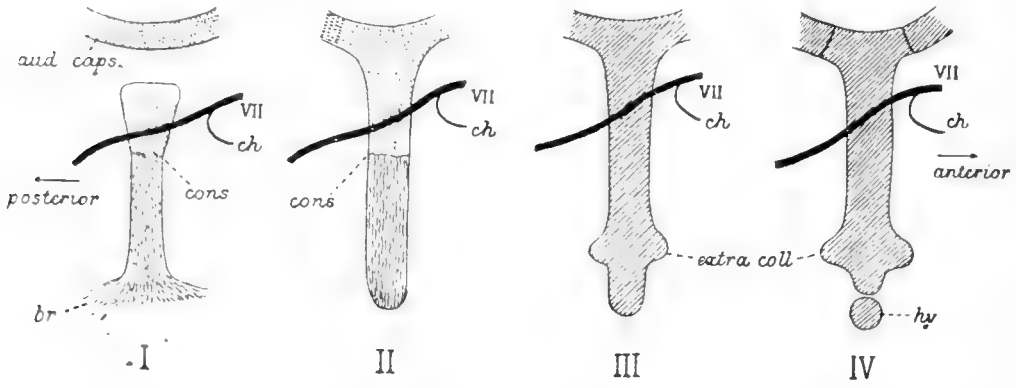
Figs. 4, 5, and 6 drawn with camera under Zeiss 4, AA.

occupy a very small part of the space subsequently occupied by the stapes, unless we imagine it bodily thrusting the hyoid arch before it, a process which is not easy to imagine in ill-defined pro-cartilaginous structures, and for which there is not the least shadow of evidence.

During the sixth and seventh days of incubation chondrification sets in. In the seven-day chick auditory capsule and hyoid are both perfectly chondrified and perfectly continuous

with one another, the constriction observable in the five- and six-day chicks having, moreover, disappeared.

In the eight-day chick the stapes is divided off from the auditory capsule, and the extra-columella is severed from the extreme distal end of the hyoid arch. This extreme end of the hyoid arch, which takes no part in the formation of the extra-columella is excessively small, only running through a few sections. My series of sections at this stage show the continuity and homogeneity of the stapes and all parts of the columella, the ossification of the stapes not occurring until a later period.



I. Five-day chick. II. Six-day. III. Seven-day. IV. Eight-day. All viewed from without. *aud. caps.* Auditory capsule. VII. Seventh nerve. *ch.* Chorda tympani. *cons.* Constriction. *br.* Branchial blastema. *extra coll.* Extra collumella. *hy.* Hyoid.

It should be plain from this account that the chondrified stages in the seven- and eight-day chicks, with the description of which previous authors have been content, really tell us little by themselves; but the previous history of the hyoid arch in the pro-cartilage condition shows (1) that the whole of the extra-columella and part, at least, of the stapes are formed from it; (2) that the derivation of the foot-plug of the stapes, and perhaps the extreme distal part of the stapedial rod may be either from hyoid or from auditory capsule, but from which of the two it is impossible to assert, since the two elements are already inextricably fused before chondrification occurs; without leaving any visible boundary between them. It would be safe to say that certain cells in

the foot-plug are derived from the hyoid arch and certain cells from the auditory capsule. The important fact, however, clearly expressed in Figs. 4 and 5 is that the dorsal part of the hyoid arch, i. e. the part lying between the seventh nerve and the auditory capsule (*stap.* in Figs. 4, 5, and 6), gives rise to part, at least, of the stapes. The meaning of the constriction in the five- and six-day chicks must remain doubtful; it corresponds in position to a division between hyomandibular and keratohyal, and to the later division between stapes and extra-columella.

The following diagrammatic reconstructions will make the foregoing observations clear.

3. Conclusion.

The value of the embryological evidence here presented is partly positive, partly negative.

Positively, it may be stated that in the chick the contribution of the auditory capsule to the columella is exceedingly small, probably confined to the foot-plug of the stapes; at any rate the main part of the stapes and the whole of the columella is formed from the hyoid arch. Negatively, it proves the futility of basing arguments upon this question on isolated stages, or on cartilaginous stages which have not been traced back to their earliest procartilaginous forerunners. Taking this into consideration the supposed derivation of the stapes of Sauropsida from the auditory capsule (**9**), and the possible interpretation of Sphenodon in this manner (**12** and **14**) becomes exceeding doubtful; in birds, at any rate, as we have seen, the condition confirms the opinion arrived at on theoretical grounds by Gaupp (**11**), that the stapes of Sauropsida corresponds to the stapes of Mammalia, and to the hyomandibular of fishes. Mammalia and Sauropsida have this much in common, that they have both converted the hyomandibular or dorsal portion of the hyoid arch into the stapes; but subsequently they have gone on different lines in evolution, the Sauropsida making use of the more ventral part of

the hyoid to complete their chain of ossicles (extra-columella), while the Mammalia have pressed into this service the constituents of the arch in front—namely, the quadrate and articular (incus and malleus).

(Since this article was in type Versluys (15) has published a most thorough account of the development of the Lacertilian columella. I am happy to see that his results are in complete accord with my own).

Literature.

1. Platner, F.—'Bemerkungen über das Quadrat-bein und die Paukenhöhle der Vögel,' 1839.

2. Hasse, C.—"Zur Morphologie des Labyrinths der Vogel," 'Anatom. Studien,' Bd. i, 1873.

3. Parker, W. K.—"On the Structure and Development of the Skull of the Common Fowl," 'Phil. Trans. Roy. Soc. Lond.,' vol. clix, pt. ii, 1869.

4. Huxley, T. H.—"On the Representatives of the Malleus and Incus of the Mammalia in the other Vertebrata," 'Proc. Zool. Soc. Lond.,' 1869.

5. Moldenhauer, W.—"Die Entwicklung des mittleren und des ausseren Ohres," 'Morph. Jahrb.,' Bd. iii, 1877.

6. Magnien, L.—"Recherches sur l'anatomie comparée de la corde du tympan des oiseaux," 'Comptes Rendus de l'Académie des Sciences,' t. ci, 1885.

7. Gadow, H.—'Phil. Trans.,' 1888, vol. clxxix.

8. Gadow und Selenka.—"Vögel," 'Bronn's Klassen und Ordnungen,' Bd. vi, Abt. 4, 1891.

9. Hoffmann, C. K.—"Reptilien," 'Bronn's Klassen und Ordnungen,' Bd. vi, Abt. 3, 1891.

10. Versluys.—"Die mittlere und aursere Ohrsphäre der Laccartilia und Rhyncocephalia," 'Zool. Jahrb.,' Bd. xii, Heft. 2.

11. Gaupp, E.—"Ontogenese und Phylogenese des schalleitenden Apparates bei den Wirbelthieren," 'Anät. Hefte,' 2te Abt., 1898.

(See this paper for discussion of whole question and complete list of literature.)

12. Schauinsland, H.—"Weitere Beiträge zur Entwicklungsgeschichte der Hatteria," 'Arch. Mikr. Anat.,' lvi, 1900.

13. Kingsley.—"The Ossicula Auditus of Vertebrates," 'Tuft's College Reports,' 1900.

14. Howes, G. B., and Swinnerton, H. H.—"Developement of the Skeleton of the Tuatara," 'Trans. Zool. Soc. Lond.,' vol. xvi, pt. i, 1901.

15. Versluys, J.—"Entwicklung der Columella auris bei den Lacertilien," 'Zool. Jahrb.,' Bd. xix, Heft 1.

Notes on Rhabdopleura Normani, Allman.

By

G. Herbert Fowler, B.A., Ph.D., F.Z.S., F.L.S.

(With Plate 3.)

These notes, written mainly some years ago, did not seem worthy of publication by themselves. But my friend Mr. Harmer lately called my attention to some remarkable statements made by Messrs. Conte and Vaney[1] which seem to justify the publication of the present paper, despite the small quantity and imperfect preservation of my materials.

These gentlemen state that the peduncle is inserted "en un point d'où divergent le corps proprement dit, l'épistome et les deux bras." This point, on the ventral surface, is the mouth; but, as a matter of fact, the peduncle is inserted considerably behind it (compare Professor Lankester's figures from living material[2]). I can neither confirm nor deny the statement that the "fibres musculaires de ce pédoncle se prolongent dans les bras et dans l'épistome," but I do not think it probable that they really extend so far; the longitudinal muscles of the peduncle are for the retraction of the animal as a whole in its tube; the graceful movements of arms and epistome, shown so beautifully in Professor Lankester's figures, demand an intrinsic musculature, parts of which I have already recorded.[3] It is stated that I "denied" the existence of the testis figured and described by Lankester,

[1] A. Conte and C. Vaney, 'Comptes rendus Acad. Sci. Paris,' cxxxv, pp. 63, 748.

[2] E. R. Lankester, 'Quart. Journ. Micr. Sci.,' xxiv, pl. 38.

[3] G. H. Fowler, 'Festschrift zum 70ten Gebürtstage, Rudolf Leuckarts, Leipzig, 1893, 4to.

whereas the original runs that "I have been unable to meet with any generative organs," my specimens not being sexually ripe. The account which the French authors have furnished leads one to await their figures of the generative organs with interest.

To say of the cœlom that "les sub-divisions indiquées par Fowler n'existent pas" is rather sweeping, in the face of the camera drawings which I furnished in my last paper on the subject; but as our authors go on to say that they have vainly sought the excretory canals and collar-pores, one begins to suspect that either the preservation of the material or the technique of the microtomist was imperfect. When we further learn, of the structure which I regarded as a probable homologue of the "notochord" of Balanoglossus and Cephalodiscus, that "cette prétendue chorde n'était autre chose que l'extrémité antérieure du pedoncle," one can only regret that these gentlemen have not already figured the way in which the latter post-oral and ventral structure gets across, or behind, or beside the mouth, so as to become continuous with the pre-oral "notochord."

I regret that I cannot draw the septa between the body-cavities more clearly than I have already done, but at least I hope that fig. 19 may convince Messrs. Conte and Vaney of the existence of the collar-canals and pores. This figure has been drawn with a camera lucida from five successive sections; the uppermost exhibiting the external opening, the next two the collar-canal, the last two the internal opening; the cell-structure is sufficiently well preserved to allow one to see that the cells are long and columnar in the canal, with the nuclei near the base of the cell; but, as the histology as a whole is not good, I prefer to represent the sections as "coupes histologiques schématiques" rather than to draw guesses at cell outlines, which are moreover wholly unimportant in this connection.

I. The Stalk of the Adult.

In a series of transverse sections the first appearance of

the insertion of the stalk is indicated by a thin crescentic plate of longitudinal muscle-fibres, which seem to form part of the somatic mesoderm of the body on the ventral surface. They are first recognisable some little distance above (anterior to) the bend of the alimentary canal. At the level of the intestinal flexure the muscle-plate has become somewhat thicker (fig. 1).

When clear of the body of the polyp, the soft part of the stalk ("gymnocaulus" of Lankester) shows the relations represented diagrammatically in fig. 2. It is presumably covered entirely by ectoderm; this ectoderm is certainly thick and glandular on the upper side, that turned towards the polyp. Beneath this lies the longitudinal muscle as two J-shaped bands separated from one another by a septum, which bisects the cavity of the stalk. At the ventral border of this septum the ectoderm is thickened into a triangle, the cells of which are not pigmented, as is the rest of the ectoderm, and stain very faintly; they have very much the appearance of a superficial nerve (figs. 2, 3, *a*). Abutting on this triangle a fine canal is excavated in the substance of the mesentery, recognisable in many sections and several specimens, but not in all; it may perhaps be an artificial structure (fig. 2, *b*). In the central part of the stalk another cavity is always visible, generally completely filled with a granular mass, but in the section figured this mass had shrunk away from the walls, which are thus rendered more conspicuous (figs. 2, 3, *end?*).

At the junction of the soft stalk with the body the relations are extremely difficult to determine, owing to the obliquity of the structures concerned and to a rotation of the stalk. The cœlom is comparatively broad at the point of insertion, and I believe that I can trace the paired cavities of the stalk into the cœlom, and the central cavity of the mesentery into continuity with the endoderm. In palliation of this uncertainty, I have drawn the outline of a human red blood-corpuscle on the same scale (fig. 2, *r. c.*), from which it may be gathered readily that the difficulty of study of such

minute objects in imperfectly preserved and limited material is considerable.

At the transformation of soft stalk (gymnocaulus) into hard stalk (pectocaulus) the high ectoderm spreads round three-quarters of the circumference, and presumably secretes the dark brown caulotheca, or stalk-pipe (fig. 3). Still further posteriorly the caulotheca invests the pectocaulus completely, the muscles disappear, and the soft tissues now consist of a central core, apparently continuous with the central (? endodermal) core of the gymnocaulus, and surrounded by a membrane; it is certainly flanked, and probably entirely surrounded, by pigmented ectoderm-cells.

As figs. 1 to 4 are all drawn in the same position as regards the polyp, it will be noticed that there is a rotation of the stalk through about 90°; the mesentery, which originally lay in the oro-anal plane of the polyp, finally comes to lie right and left as regards the polyp-axis, although dorso-ventral as regards the colony. This may be accidental (as Mr. Harmer suggests), but is at any rate not unusual.

II. The Anatomy of a Bud.

The specimen which I select for description was apparently at a stage intermediate between Nos. 6 and 7 of Professor Lankester's fig. 3, pl. 39, in that the lophophoral arms were longer than in No. 6, but had not yet begun to develop filaments. It has been drawn as fig. 18 of this paper. The proboscis or epistome is large, the collar region small and only slightly larger than the trunk, the trunk indistinguishable externally from the gymnocaulus. At this stage, therefore, the long axis of the body is a continuation of that of the gymnocaulus—a condition unlike that of the adult (cf. Lankester, op. cit., pl. 37, fig. 1).

As to the lophophoral arms and upper part of the proboscis, there is nothing of special developmental interest to say; the arms simply grow out from the collar region, and contain off-sets of the collar body-cavity from the beginning.

Figs. 5 to 14 are from a continuous series of successive sections, all of which are drawn; it is therefore possible to follow the anatomy minutely. The sections are slightly oblique. Starting with fig. 8, there seems to be a well-marked stomodæum, which, owing to the obliquity of the sections, appears erroneously to open on the right side only. This stomodæum is sharply separated from the upper (rectal) part of the alimentary canal by a stout membrane; the canal itself at this level appeared to be a vacuolated mass, in which no epithelial-cell outlines were recognised. All three subdivisions of the cœlom were represented in this section—a small part of the proboscis-cavity (*bc.*[1]), the left collar-cavity (*bc.*[2]), and the trunk-cavity, apparently divided into two parts by the alimentary canal dorsally and ventrally (*bc.*[3]). On the animal's right side the section passed nearly along the septum between the collar- and trunk-cavities.

In the section above this (fig. 7) the collar-cavity of the right side appeared, and the trunk-cavity of that side had almost vanished. The next section upwards (fig. 6) was unfortunately folded between proboscis and cœlom, so that not more than has been drawn could be recognised; it was obvious, however, that the stomodæal groove of the previous section had been folded off as a rod, which contained (I think) a cavity. In the highest section figured (fig. 5) the alimentary canal was no longer met with; the rod of the previous section was in the position of the notochord.

Passing downwards from fig. 8, the next section (also folded at the attachment of the proboscis) showed a thick muscle-band on the outer wall of the right-hand half of the trunk body-cavity, other structures remaining much as before (fig. 9). In fig. 10 the stomodæum had entered the alimentary canal (*œ*), and the lower lip had been reached. In fig. 11 the right trunk body-cavity had increased considerably in size, and the attachment of the proboscis had been passed. The left collar-cavity had all but disappeared in fig. 12; the left trunk-cavity showed its longitudinal muscle, and a septum separated the two trunk-cavities ventrally. In fig. 13 the

alimentary canal began to diminish, the mesentery to elongate; and in fig. 14 the alimentary canal appeared to be represented by the central core of the mesentery of the gymnocaulus, the two trunk-cavities becoming the paired cavities of the stalk.

I have endeavoured to express my interpretation of these sections by an imaginary longitudinal section in fig. 15. If my views are correct, two things follow—that the notochord in the bud is of ectodermal origin, and that the gymnocaulus contains all three embryonic layers, the proliferation and growth of which give rise to equivalent structures in the adult.

As regards the notochord, I have long suspected that it was a stomodæal structure in Balanoglossus and Cephalodiscus, and there can be little hesitation in assigning it to the ectoderm in buds of Rhabdopleura on the strength of these sections. Figs. 7, 8, and 9 show an epithelial invagination below the proboscis-stalk, which, from the character of the cells, is fairly certainly ectodermal, and is continuous with the so-called notochord; the alimentary canal, on the other hand, appears, so far as I can see, to be syncytial and vacuolated rather than epithelial; this is shut off by a basement membrane from the stomodæum at the plane of these sections, and is presumably the future endoderm.

As regards the structure of the adult gymnocaulus, I have no personal doubt of the view given above, that the contents of the central cavity in the septum are continuous with the alimentary canal of the adult, and give rise to the alimentary canal of the bud; they are presumably of endodermal origin. Similarly the paired cavities of the gymnocaulus are traceable fairly unmistakably into the trunk-cavities of the bud, less certainly into those of the adult. At the same time, the structures in question are so minute that these views have only the value of a personal conviction, and require confirmation from other sources.

These notes and drawings of the structure of the stalk and bud, such as they are, were made before the publication of

Dr. Masterman's paper[1] on the budding of Cephalodiscus, but I am unable to bring the two sets of observations into accord. There is no doubt that Masterman's picture of the stalk in Cephalodiscus is correct in exhibiting two cavities bounded by a thickish membrane (as in his pl. i, fig. 18), whatever may be the correct interpretation of these structures. There is equally no doubt that my fig. 2 is also correct (interpretations excepted) in showing the cœlom of the stalk divided completely by a septum. But Masterman interprets the cavities in Cephalodiscus as "blood-" sinuses, whereas my specimens lead me to believe that the central core of the Rhabdopleura septum is continuous with the lining of the alimentary canal. Unfortunately buds smaller than that described in detail above proved to be too minute to allow of definite conclusions being drawn,[2] and the preliminary remarks of MM. Conte and Vaney are too brief and vague to settle the matter (op. cit., p. 749).

Cephalodiscus and Rhabdopleura agree in the precocious formation of the epistome, in the continuity of the stalk-cœlom with that of the bud, and in the presence of a nerve-like stripe of ectoderm on the stalk.

EXPLANATION OF PLATE 3,

Illustrating Dr. G. Herbert Fowler's "Notes on Rhabdopleura Normani, Allman."

Note.—As in my previous paper (op. cit. supra), the trunk-cœlom has been drawn all round the alimentary canal on the authority of Prof. Lankester's observations on living specimens, although in my shrunken specimens it is

[1] A. T. Masterman, 'Trans. Roy. Soc. Edin.,' xxxix, p. 507.

[2] At the same time, the structures are large enough to allow of accurate determination in material specially preserved; mine had been roughly preserved (apparently merely in strong alcohol), for the sake of the Lophophelia on which it grew; as it was "Challenger" material, thirty years' preservation has not improved it.

only visible here and there; this has necessitated a slight re-adjustment of the comparative thicknesses of the body-layers in the figures. The ectoderm has in many figures been drawn thicker than it actually appears. In my depigmented specimens it is invisible over a large part of the body and stalk. With the exception of fig. 15, all outlines have been drawn with the Abbé camera lucida. Fig. 15 is based on a plotting of the actual section-drawings on scaled paper, free-hand curves being drawn through the points thus obtained; the horizontal scale is therefore nearly correct, the vertical scale arbitrary, but estimated roughly on the thickness of the sections.

Reference Letters.

a. Streak of unpigmented ectoderm in the gymnocaulus (? nervous). *al.* Alimentary canal. *asc.* Ascending half of the alimentary canal. *b.* Space in the mesentery (? blood-vessel or artificial). *bc*^{1}. Cœlom of the proboscis or epistome. *bc*^{2}. Cœlom of the collar region. *bc*^{3}. Cœlom of the trunk or body region. *caul.* Caulotheca, or stalk-pipe. *c. c.* Collar-canal. *d. mes.* Dorsal mesentery. *desc.* Descending half of the alimentary canal. *ect.* Ectoderm. *end.* Endoderm of the adult. *end. ?*. Core of the mesentery, probably endodermal. *mes.* Mesentery or septum of the gymnocaulus. *musc.* Longitudinal retractor muscle. *n.* Dorsal thickening of ectoderm (? nerve-plate). *nch.* Stomodæal diverticulum (so-called notochord). *œ.* Œsophagus. *pr.* Proboscis. *r. c.* Outline of a human red blood-corpuscle, for scale. *s.* Septum between the body-cavities of the proboscis and collar. *st.* Stomodæum. *tub.* Tubarium. *v. mes.* Ventral mesentery.

PLATE 3.

Figs. 1—4 relate to the stalk of the adult.

Fig. 1.—Section of the posterior end of the adult, at the point of flexure of the intestine, showing the continuation of the longitudinal muscle of the stalk on to the body. × 430.

Fig. 2.—The gymnocaulus, below the body of the animal. × 820.

Fig. 3.—The gymnocaulus, at the commencement of the pectocaulus. × 820.

Fig. 4.—The pectocaulus. × 820.

Figs. 5—14 are successive sections of the bud drawn as fig. 18. The plane of section is somewhat oblique and the epistome twisted. × 520.

Fig. 5.—Below the attachment of the lophophoral arms.

Fig. 6.—Through the highest point of the alimentary canal, dorsally. No anus was visible.

Fig. 7.—The stomodæum, open on the right side.

Fig. 9.—The right longitudinal muscle of the stalk appears.

Fig. 10.—The œsophagus separated from the stomodæum by the lower lip.

Fig. 11.—Below the proboscis-stalk.

Fig. 12.—The left longitudinal muscle of the stalk appears.

Fig. 14.—The gymnocaulus.

Fig. 15.—Diagrammatic reconstruction of the foregoing sections as a longitudinal section beginning just below the insertion of the lophophoral arms, the outline of the trunk body-cavity, which of course is not cut in a median dorso-ventral section, being marked by dashes. The numbered arrows indicate the corresponding figures of the transverse sections.

Figs. 16, 17, 18.—Buds at the end of a terminal branch, a short length of pectocaulus intervening between the successive figures. Of these fig. 16 is the growing end of the branch, and fig. 18 the oldest bud drawn. × 140. The lowest bud in Fig. 16 is viewed from the right side, and gives a good idea of the way in which the lophophoral arms spring from the end of the body proper, and the proboscis stands out on the ventral side.

Fig. 19.—Successive sections of the collar-pore and canal of the right side of an adult animal. × about 520.

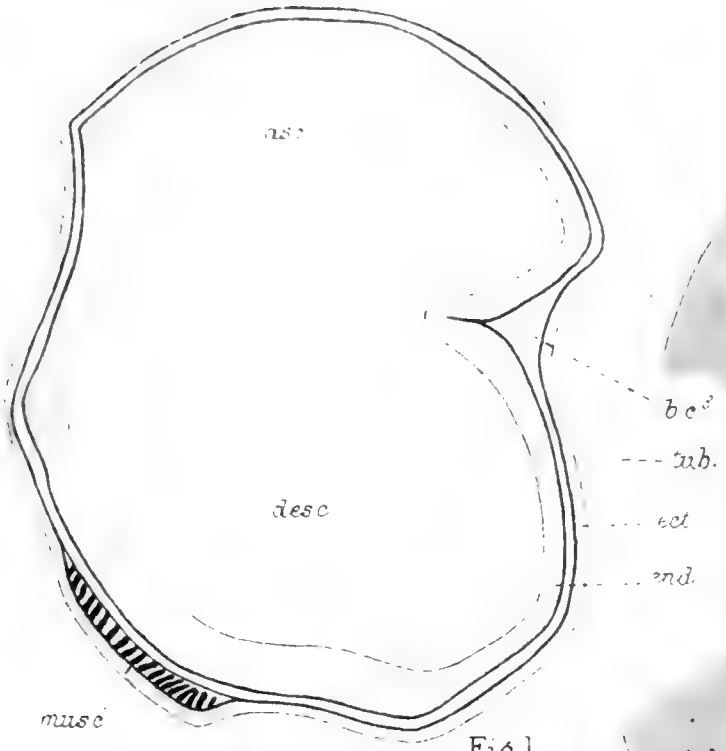


Fig. 1.

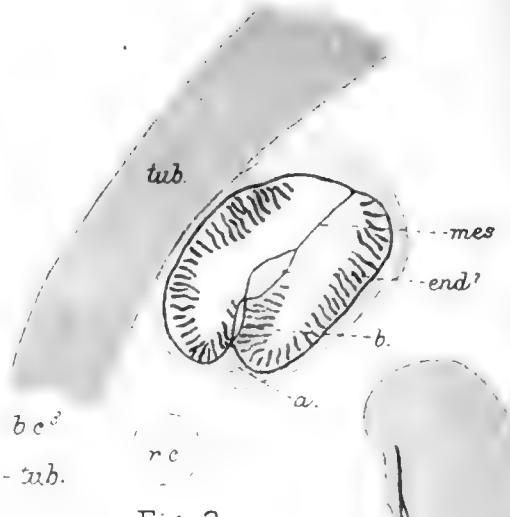


Fig. 2.

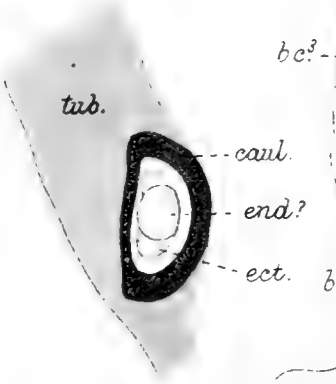


Fig. 4.

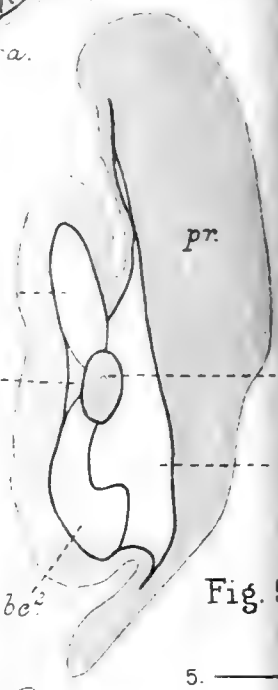


Fig. 5.

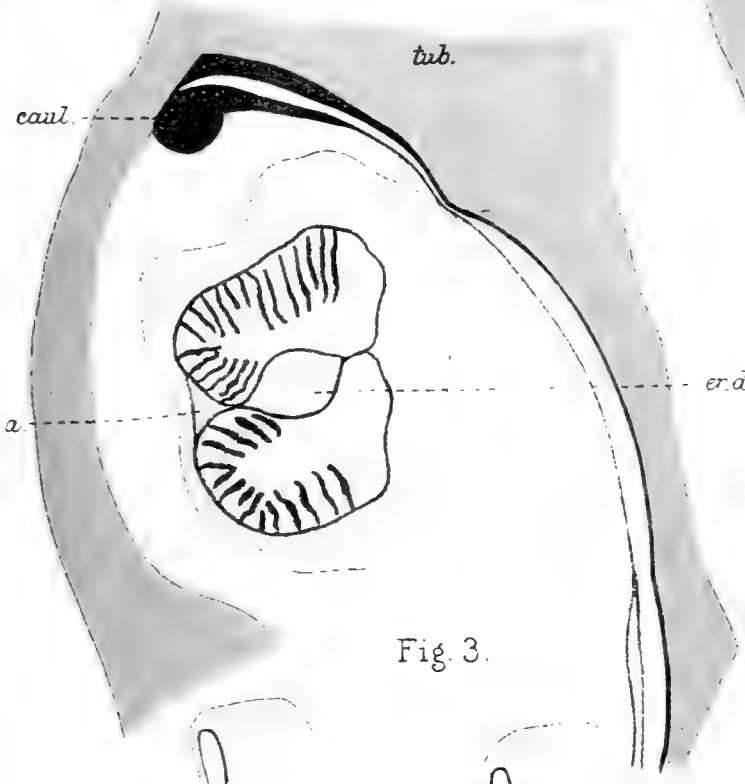


Fig. 3.

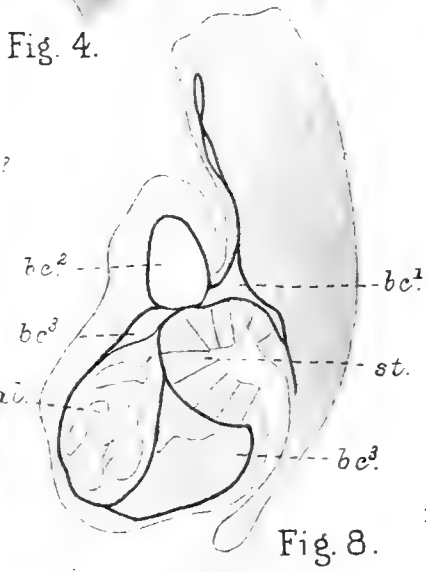


Fig. 8.

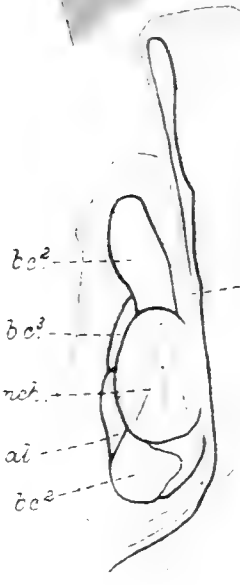


Fig. 6.

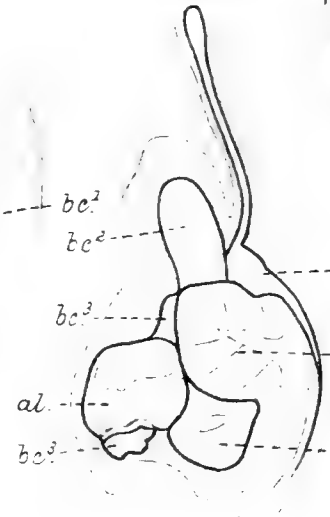


Fig 7.

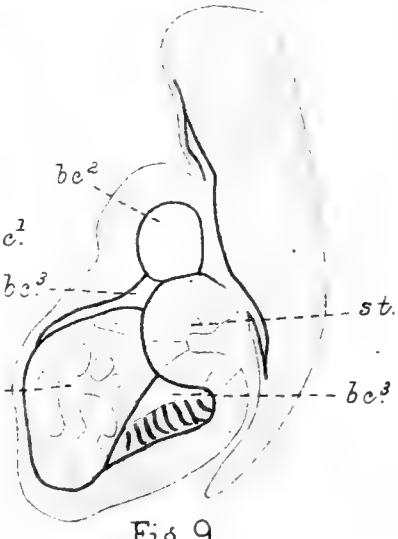


Fig. 9.

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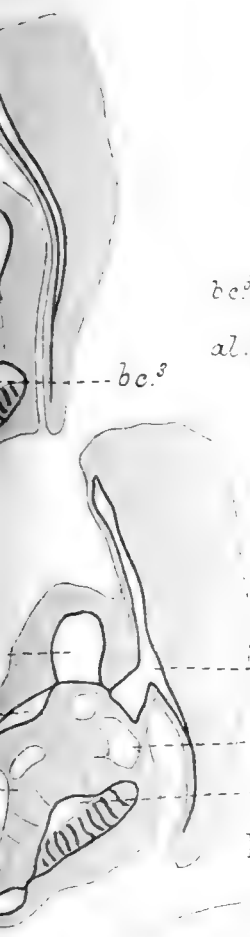


Fig.10.

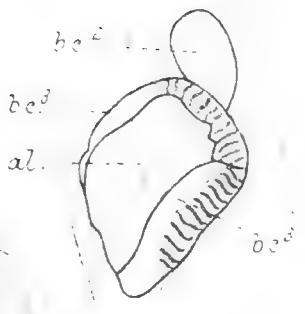


Fig 12.

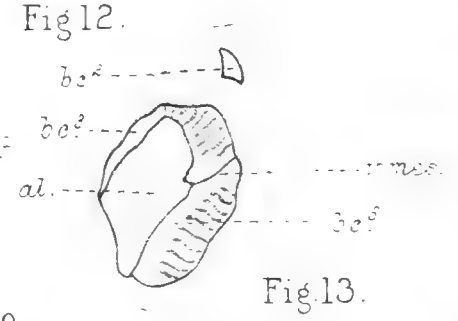


Fig.13.

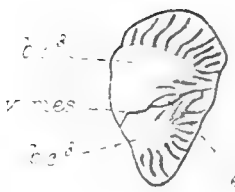


Fig.14.

Fig.17.

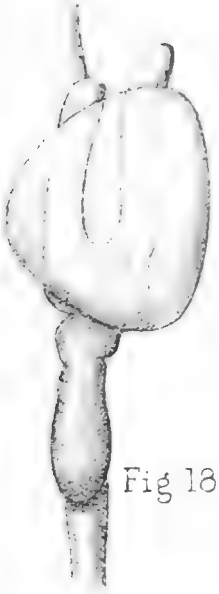
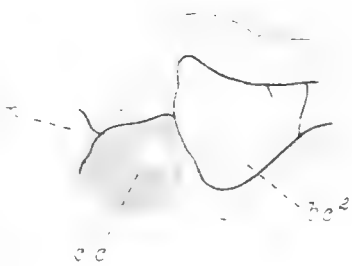


Fig 18.



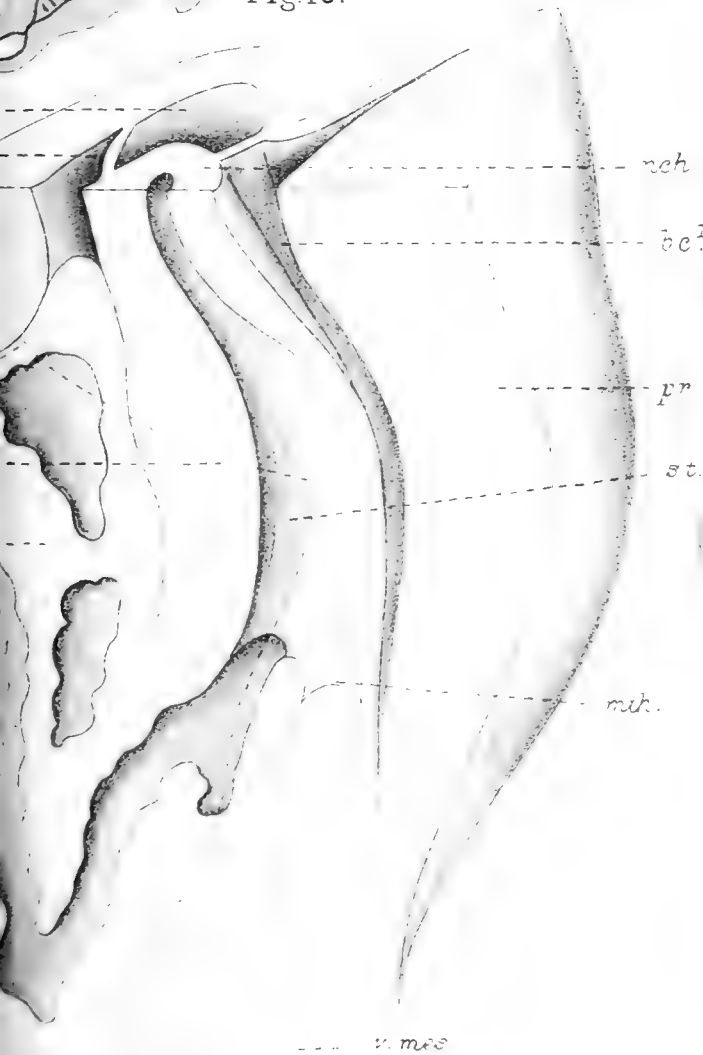


Fig 15.

Fig.16.

Fig.19.

Some Observations on the Anatomy and Affinities of the Trochidæ.

By

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(From the Zoological Laboratory, Royal College of Science, London.)

With Plates 4, 5, and 6.

The results embodied in this paper are the outcome of a series of observations on the anatomy of various species of Trochus. It was my original intention, acting on the suggestion of the late Martin F. Woodward, to confine my attention mainly to one species, viz. Trochus magus, and study that as a type form. I was unaware at that time of the existence of a memoir on Trochus, published in the 'Zoologie Descriptive' (38), in which a very adequate account of the anatomy of Trochus turbinatus (Born) is given by A. Robert. As this article gives a sufficiently detailed account of the anatomy of a fairly typical form, it will be unnecessary for me to give more than a general résumé of the main points of the anatomy, but rather to amplify any features that have not been fully described, and to point out any differences that may exist in the organisation of the various species which I have been able to examine, noting whether these differences are sufficient to justify the existence of the numerous sub-genera into which the genus Trochus has been divided upon conchological grounds.

All the species which I have so far examined are British,

the greater part of them having been obtained from Plymouth during the months of July and August, 1901.

For specimens of Trochus exasperatus and T. Montacuti I am indebted to Mr. E. R. Sykes, and of T. magus to Mr. E. W. Holt.

I wish here to express my best thanks to the Committee of the Royal Society for a grant which enabled me to carry on my researches at Plymouth, also to the British Association and Zoological Society for the use of their tables at the Marine Biological Laboratory during July and August, 1901.

The various species of the genus Trochus of which there are a considerable number, have been grouped into numerous sub-genera. These sub-divisions have been founded upon conchological differences without regard to the anatomical organisation of the animal. It is highly probable that if anatomical characteristics are taken into account the number of sub-genera can be considerably reduced.

The following species of Trochus are those which I have examined :

1. T. magus (Linn.).
2. T. cinerarius (Linn.).
3. T. umbilicatus (Montagu).
4. T. tumidus (Montagu).
5. T. lineatus (Da Costa).
6. T. zizyphinus (Linn.).
7. T. granulatus (Born).
8. T. striatus (Linn.).
9. T. exasperatus (Pennant).
10. T. Montagui (Gray).

These species are, according to Forbes and Hanley (**17**), grouped into two sub-genera, viz. 1—5 under the sub-genus Gibbula and 6—10 under the sub-genus Trochus.

If we follow the classification given either by Gwyn Jeffries (**24**) or Tyron (**43**), we must group the above species into three subgeneric divisions, viz. 1—4 under the sub-genus Gibbula (Leach), 5 under the sub-genus Monodonta (Lamarck), or Trochocochlea (Klein), and 6—10

under the sub-genus Calliostoma (Swainson), or Zizyphinus (Leach). According to the latter system we have the species Trochus lineatus included in a separate sub-genus, Trochocochlea (Klein), which species is the only British representative of the sub-genus, though there are numerous exotic species belonging to it. This separation of T. lineatus from the sub-genus Gibbula, in which it is placed by Forbes and Hanley, is based upon conchological grounds which to my mind do not seem to be of sufficient importance to justify it, though my own observations are based upon the examination of a single species, T. lineatus.

The characters of the three sub-genera Gibbula, Trochocochlea, and Calliostoma are given by Jeffries (**24**) as follows:

1. Gibbula (Leach).—Shell low spired and umbilicate.
Examples: T. magus (Pl. 4, fig. 1).
T. cinerarius (fig. 2).

2. Trochocochlea (Klein).—Spire moderately raised, base slightly umbilicate in the adult and perforated in the young, pillar lip furnished with a strong tubercular tooth.
Example: T. lineatus (fig. 3).

2. Calliostoma (Swainson).—Spire pyramidal, base imperforate, pillar lip notched or angulated at the lower part.
Example: T. zizyphinus (fig. 4).

Apparently the only difference in the characters of the sub-genera Trochocochlea and Gibbula is in the height of the shell, the absence of an umbilicus, and the presence of a tooth on the pillar lip. But these characteristics are not necessarily confined to the sub-genus Trochocochlea, for species of Gibbula may occasionally be imperforate or high spired (T. cinerarius, fig. 3). As Gwyn Jeffries remarks (**24**, vol. iii, p. 294), "The shells are usually low spired and deeply umbilicate, but varieties of T. tumidus, T. umbilicatus, and T. cinerarius have the spire raised. Again, T. lineatus is the only representative of Klein's genus Trochocochlea, in which the spire is raised, the base imperforate, and the pillar lip furnished with a blunt tubercle or notch; the last

two characters are common, however, to several species of Gibbula and the typical section Zizyphinus, which last has a pyramidal spire. It is also not generally known, but not less the fact, that young shells of T. lineatus (the type of Trochocochlea) are always deeply umbilicate."

We see, then, that the conchological differences between the two sub-genera are very meagre and valueless for diagnosis; and when we come to compare their anatomical structure, we find they are so nearly identical that it seems quite unnecessary for the separate sub-genus to be retained.

The species 6—9, however, fall into a group quite distinct from that of Gibbula, and exhibit anatomical differences that warrant their separation into a sub-genus, viz. Calliostoma. Here, however, although T. zizyphinus and T. granulatus are very different in many respects from any species of Gibbula, some of the smaller species of Calliostoma, viz. T. striatus, and T. exasperatus, present points of startling similarity in the radula and some external features to T. magus and other species of Gibbula. They, however, in possessing pyramidal shells, and in the presence of an accessory structure in connection with the female genital organs (a structure common to all the British species of Calliostoma which I have examined), undoubtedly belong to this latter sub-genus.

External Characters.—The head is moderately large, and is bent downwards into a cylindrical snout, on the under-surface of which is situated the mouth. There are present on either side of the head three appendages, the outermost of these, the ocular peduncles (figs. 5, 6, 7, *oc. p.*) are short, laterally flattened structures, presenting in cross-section a somewhat oval contour. Near the apices of these the eyes are situated. Internal to the ocular peduncles are placed the cephalic tentacles, highly muscular organs, capable of great extension and covered externally with fine cilia (fig. 7, *c. t.*)

An interesting condition is seen in the larval forms of Trochus (vide Robert, **38**, fig. 508, K)— the cephalic tentacles

are branched at their extremities, thus presenting an appearance similar to that described by Woodward in the cephalic tentacles of Pleurotomaria (**45**, pl. 13, fig. 1). In none of the adult specimens of Trochus examined have I noticed an indication of this branching, even as an abnormality, though one specimen of T. zizyphinus exhibited a most peculiar and interesting abnormality, in that on the right ocular peduncle three eyes were present in place of the usual one. The left eye was perfectly normal.[1]

The third pair of appendages present on the head of the Trochidæ are the cephalic lappets (figs. 5, 6, 7, *c. l.*) These structures are very variable in size: in those species belonging to the sub-genus Gibbula they are large and conspicuous, their free margins being fringed and ciliated; whilst in T. zizyphinus and other species belonging to the sub-genus Calliostoma they are extremely small and sometimes entirely absent. In connection with the ocular peduncles there is a most remarkable little organ existing in many of the species of Trochus, viz. a small pointed appendix situated underneath and behind the right ocular peduncle (fig. 5, *a. oc. p.*) In T. cinerarius (Pelseneer, **36**, pp. 46, 47) and T. umbilicatus it is comparatively large, and can easily be found. It is present in T. magus and T. lineatus, though much smaller than in the preceding species, and is noticeable only as a small protuberance on the ocular peduncle. Clarke (**11**, p. 313) has described a similar appendix in T. tumidus as a penis, though in the three specimens of this species which I examined I was unable to find any trace of the structure. In the sub-genus Calliostoma it is variable in its appearance or non-appearance: T. zizyphinus and T. granulatus are entirely without it, while in T. striatus and T. exasperatus, though small, it is usually present. It is not confined to the Trochidæ, but is present in other genera, viz. Crepidula, Capulus, and Calyptrea, being especially well developed in the last genus. It has been regarded by several observers as being of the nature of a penis, but in

[1] Vide 'Nature,' No. 1693, vol. lxv, p. 535, April 10th, 1902.

Trochus at any rate it has undoubtedly nothing whatever to do with the genitalia; at least it is not of the nature of a penis, because when present it is found in both male and female. Besides, it is a solid organ and exhibits no trace of canal or groove which might serve for the transmission of sperms, and were it of this nature we should expect to find it in all species, and not, as is actually the case, present in some and absent in others. Those species in which it occurs are mainly littoral forms, and there appears to be some correlation between its presence and the existence of a certain asymmetry that occurs in the epipodial lobes of these.

The foot is a large muscular organ, capable of great extension; it is beset on its lateral surfaces with numerous papillæ, giving it a rugose appearance. The anterior margin presents in some species, T. granulatus, etc. (fig. 6), a large tranverse groove separating the sole from the upper part of the foot. A similar groove occurs in Pleurotomaria and many other Gasteropods; it is evidently of importance, though its function is somewhat enigmatical. In the Trochidæ it is present only in those species belonging to the sub-genus Calliostoma, and is not represented in any of the Gibbulæ which I have had the opportunity of examining. When present this groove leads into a large tubular pedal gland (fig. 6, *p. gl.*), which extends some distance into the anterior portion of the foot; the gland is composed of large deeply staining cells, containing granular protoplasm and rather small nuclei. The canal of the gland is lined with ciliated epithelium. Houssay has described a similar, though slightly more complex gland in Trivia Europæa (**23**, pp. 272—275, pl. xiv, fig. 2), in which a large transverse groove is present on the anterior margin of the foot, which leads into a longitudinal ciliated canal surrounded by cells of the pedal gland. In cross-section the pedal gland presents a similar appearance to that of Chenopus as figured by Houssay (**23**, pl. xiii, fig. 4, pp. 278—281).

Though there is no definite pedal gland in any of the species of the sub-genus Gibbula, such a structure is not

entirely unrepresented, but takes the form of a number of large unicellular gland-cells on the under surface of the foot, aggregated more especially round its anterior margin. Although Pleurotomaria has the transverse groove on the anterior margin of the foot very well developed, there is no longitudinal canal or pedal gland connected with it, such as exists in T. zizyphinus, etc, but it is more than probable that the groove contains numerous gland-cells.

On the dorsal surface of the foot there is invariably present a specialised area running from the opercular lobe to the posterior extremity. The exact appearance of this differs somewhat in the different species. In T. granulatus (fig. 8) and T. zizyphinus it is well defined and **V**-shaped, bounded by two lateral converging furrows. A shallow median furrow, together with the two lateral furrows, arise from under the free border of the opercular lobe and run down the dorsal surface of the foot for a short distance; the median furrow then terminates, and numerous transverse grooves make their appearance and are continued to the end of the foot, the posterior grooves being deeper than those more anterior. These grooves are not continued right across the foot from side to side, but are bounded by the two converging lateral furrows. In addition to these deep transverse grooves there are numerous smaller branching furrows which run in a transverse direction across the dorsal surface of the foot from side to side; these are not interrupted by the lateral furrows. In the remaining species there is a slight difference in the arrangement of this specialised portion of the foot. The lateral furrows are only continued for a short distance beyond the opercular lobe and do not limit the transverse furrows to a markedly **V**-shaped area.

These transverse furrows run right across the foot to the epipodial lobes and frequently branch. In Trochus magus (fig. 9) this condition is well exhibited; at the posterior extremity of the foot a clearly defined median groove is present; in T. cinerarius this median groove is continued from the opercular lobe to the extreme tip of the foot.

Similarly modified areas occur on the dorsal surface of the foot of Pleurotomaria (**45**, p. 219), and Haliotis (**44**, pp. 335, 336). This specialised area is undoubtedly glandular in nature, as, when microscopically examined in section, numerous goblet-cells are seen to exist. The epithelium covering the folds of the grooves consists of large cylindrical, ciliated cells with granular contents and large rod-shaped nuclei. Interspersed between the ciliated cells are mucous-discharging goblet-cells. Underneath this specialised area of the foot the various blood-sinuses are particularly large and numerous. No definite function has as yet been assigned to this organ, though it is without doubt in part a mucous gland; and Wegmann (**44**) has observed in living specimens of Haliotis the secretion of a mucous thread from this area. On the antero-dorsal surface of the foot is situated the opercular lobe (figs. 8, 9, *op. l.*), which is bean shaped, having its posterior margin free.

The ciliation which is so marked on the cephalic tentacles is continued over the great part of the foot, the cilia on the margin of the foot being especially long (fig. 7).

The epipodium is well developed in the Trochidæ, though more conspicuously so in the members of the subgenus Gibbula than in those of the Calliostomæ. It originates close to the ocular peduncle (figs. 5, 6, *ep. c.*) and extends to the posterior limit of the foot, attaining its maximum development in the region of the neck, where it enlarges into a cervical lobe (*ep. c.*) In the species of Gibbula the cervical lobes are asymmetrical, the right being larger than the left and having its free margin entire, while the margin of the left lobe is digitate and covered with sensory papillæ. This fringing of the left cervical lobe is very conspicuous in T. lineatus (fig. 7, *ep. c.*), also in T. cinerarius and T. umbilicatus, whereas in T. magus, though the right and left lobes are asymmetrical as regards actual size, the fringing of the left is by no means so obvious as in the preceding species, in some specimens scarcely any trace of unevenness in marginal outline being

apparent. On the other hand, in those species belonging to the sub-genus Calliostoma the right and left cervical lobes of the epipodium are perfectly symmetrical, their margins being entire and free from pectinations. According to Pelseneer (**36**, p. 46) the lobes during life are rolled up on themselves, forming channels leading into the mantle-cavity, and serving to convey water into and out of it.

The epipodium is furnished on either side with three or more tentacles, which can be extended to a considerable length. They are highly muscular, and present a great similarity in structure to the cephalic tentacles, and, like these, are covered externally by numerous fine cilia (fig. 7, *ep. t.*). The number of these tentacles is very constant in the two sub-genera; in Gibbula there are always three on each side, whilst in Calliostoma either four or five are present, but always more than three. At the base of these tentacles are situated some small appendices, the epipodial papillæ (fig. 7, *ep. p.*), which either vary slightly in shape and occasionally in number in the different species, or may be entirely absent, as in T. zizyphinus and T. granulatus. In T. cinerarius they are club-shaped structures; in T. magus they show a tendency to branch, whereas in T. lineatus they are wart-like projections at the base of the tentacles. They are undoubtedly sensory in function, probably tactile, and are innervated by the nerve going to the epipodial tentacle. In section they exhibit a slight concave depression at the apex, the epithelium lining this concavity consisting of elongated cells occasionally pigmented.

These structures have been regarded as accessory eyes, but it is extremely doubtful if they are other than tactile organs. In addition to the papillæ at the base of each epipodial tentacle there is a similar organ under each cervical epipodium, totally unaccompanied by any sensory tentacle. These anterior papillæ exhibit exactly the same structure as those previously mentioned, and though there is usually one present on either side, two or even three may be present on one side (generally the left) and one on the other.

It is of considerable interest to note that in T. zizyphinus and T. granulatus the entire absence of sensory papillæ at the base of the epipodial tentacles and under the cervical lobes of the epipodium is correlated with the perfect symmetry of the cervical lobes and the absence of an appendix on the right ocular peduncle. In the following species:—T. striatus and T. exasperatus,—which are included in the sub-genus Calliostoma,—the cervical lobes are symmetrical, but sensory papillæ are present under these lobes and also at the base of the tentacles, and, in addition, the appendix at the base of the right ocular tentacle occurs. Moreover the specialised glandular area on the dorsal surface of the foot more nearly resembles the condition seen in T. magus than the V-shaped area in T. granulatus.

The operculum is a circular, multispiral, chitinous disc with a central nucleus; the whorls overlap each other and are marked in a radial direction by numerous striæ indicating lines of growth. It differs slightly in the two sub-genera, both in colour and also in the number of whorls composing it. In Gibbula it is dark brown, and the whorls, which are fewer in number than in Calliostoma, range from six and a half to seven in adult specimens of T. magus (fig. 10), to ten or twelve whorls in T. umbilicatus and T. lineatus. The lines of growth are very distinct, and on the under side of the operculum a bean-shaped scar (fig. 10, *m. ins.*), situated eccentrically, marks the area of attachment of the operculum to the columella muscle and opercular lobe of the foot. In Calliostoma the operculum is of a light yellow colour, the volutions are more numerous, ranging from thirteen to fourteen in T. striatus, T. exasperatus, and T. granulatus to as many as fifteen or sixteen in T. zizyphinis (fig. 11). In this latter species the lines of growth are very close together, and are more distinct on the outer half of the whorl. The area of the muscle attachment is more or less triangular in shape.

The Pallial Complex.—The mantle is thin walled, with the free edge slightly thickened and occasionally plicated.

Very small and inconspicuous papillæ occur on the margin. The mantle completely encircles the body, but the posterior portion (fig. 40, *m. a.*) is very small, its margin being thin. This part of the mantle is closely attached to the columella muscle.

The mantle-cavity is large, and is divided by the gill-septum into two chambers, a large right chamber, into which the excretory and anal orifices open, and a much smaller left (dorsal) one, which encloses the lamellæ of the left side of the gill.

The gill (figs. 39—43, *g.*), is characteristically bipectinate, the gill-axis or septum bearing on either side a series of triangular gill-plates or lamellæ. This septum is attached to the mantle-wall along two lines of insertion, on the left side the attachment is near the junction between the mantle and left body-wall, whilst the other line of insertion of the gill-septum is near the mid-line of the roof of the pallial chamber. The gill, and consequently the septum, extends to the posterior extremity of the mantle-cavity, thus dividing it into the two chambers previously mentioned. The afferent and efferent blood-vessels of the gill are situated on the dorsal and ventral sides respectively of the gill-septum.

The anterior extremity of the gill is free, and is supported by a rod-like structure of cartilaginous consistency.

The gill-lamellæ are not equally well developed on both sides of the septum, those on the inner (left) side are much smaller than those on the outer (right).

The microscopic structure of the gill and gill-lamellæ of Trochus is so essentially similar to that of Pleurotomaria that it will suffice to refer to Woodward's paper on that genus (**45**, pp. 223—226) for a detailed account.

The hypobranchial gland occupies the customary position between the rectum and afferent border of the gill. Various degrees of differentation are presented in the different species. In T. cinerarius and T. umbilicatus the gland is comparatively small, in T. magus (fig. 41, *m. g.*) it is much better developed, and the glandular tissue covers the trans-

verse pallial vein (*t. p. v.*), extending up to, and a little way beyond the orifice of the left kidney; a moderately sized mucous gland is present in T. (Monodonta) monodon (Bernard, 2, p. 324). In T. zizyphinus (fig. 43) the hypobranchial gland is lozenge shaped, and the mucus-secreting cells are thickly distributed over the transverse pallial vein and the vessels uniting with it. Out of the species examined the hypobranchial gland is largest in T. lineatus, where it extends from the transverse pallial vein to within a short distance of the thickened edge of the mantle.

In all the species the main portion of the mucous gland is situated on the left side of the rectum, but there is present a small lobe on the right side. This right lobe is also larger in T. lineatus than others of the species examined.

The presence of a right lobe is of great interest when considering the asymmetrical condition of the pallial complex of Trochus. We have, again, the case of an organ situated on the right side of the body, which has, owing to the effects of dextral torsion, become very much reduced, and following in the wake of the right gill, which in Trochus has been completely suppressed. That this is so is evidenced by comparing it with Pleurotomaria (**45**, p. 228), in which a large hypobranchial gland consisting of both right and left lobes situated on either side of the rectum is present. Here the right lobe, like the right gill, is smaller than the corresponding structure on the left side, thus foreshadowing the ultimate reduction and suppression which occurs in the Azygobranchiate Diotocardia.

Béla Haller (**19**, p. 28, note) regards the reduced right lobe of the mucous gland of Trochus as the remains of the right gill which has atrophied; but when we consider that in Pleurotomaria there is present, co-existing with a functional right gill, a well-developed right lobe of the mucous gland to which the reduced right lobe in Trochus is undoubtedly homologous, the fallacy of Haller's supposition becomes apparent.

The excretory organs of Trochus have been very

adequately described by Perrier (**37**, pp. 118—131) in his admirable memoir on the kidneys of Prosobranchs. There are two kidneys present in this genus, though one only, the right, functions as a true depuratory organ. The left kidney, or papillary sac (figs. 39, 43 and 49), is an oval body situated on the left side of the rectum at the posterior end of the mantle-cavity, where it abuts on the pericardium. It communicates with the exterior by a slit-like aperture (*l. k. a.*) at its anterior end. The walls of the papillary sac are thick, and when opened are seen to be covered with numerous filiform papillæ, which in section are found to be made up of a thick layer of connective tissue traversed by a central or axial cavity which functions as a blood-space. The connective tissue is covered externally by a layer of small, ciliated, epithelial cells. This kidney is placed in communication with the pericardium by means of a long reno-pericardial canal (figs. 34, 48, *r.' p. c.*) which runs longitudinally but somewhat obliquely from the anterior angle of the pericardium along the floor of the papillary sac. The aperture in the pericardium is large and very easily discernible, and is situated on the left side of the rectum.

The aperture leading into the kidney is much smaller and is ciliated (fig. 34, *r.' p. c.*). This figure, which represents a longitudinal section through the left reno-pericardial canal of T. magus, is somewhat diagrammatic, and has been reconstructed from serial sections, the entire passage of the canal from the pericardium to the kidney occupying some fifteen sections, each having a thickness of 10 μ.

The right kidney (figs. 39, 40, etc., *r. k.*) is seen without dissection as a narrow band of tissue extending between the pericardium and the stomach and liver. It is differently coloured in the various species, being most generally of a yellowish-green colour, though in T. zizyphinus it assumes a rose-pink tint; and in this case the excretory granules present in the constituent cells have the same colour when living tissue is examined, though in material which has been preserved in alcohol they always present a greenish appearance.

The right kidney is much larger than it appears to be from a superficial examination; it extends ventrally underneath the pericardium, and approaches very closely to the left kidney, though there is no trace of communication between the two. There are slight differences in extent of this kidney in the various species, and it is most highly developed in T. zizyphinus (fig. 49) and its allies. Here the kidney can be divided into a large posterior lobe (*p. r. k.*), present in all species, and a smaller anterior lobe (*a. r. k.*) lying underneath the œsophagus, and extending almost as far as the transverse pallial vein; this anterior lobe is very feebly represented in T. magus, and almost, if not entirely, absent in T. lineatus. In Turbo, Haliotis, and Pleurotomaria the anterior lobe is very large, and forms quite a conspicuous feature of the right kidney.

T. zizyphinus, in possessing a moderately well-developed anterior lobe, approximates in this respect very closely to Pleurotomaria. The posterior lobe (*p. r. k.*) is by far the largest and most important part of the kidney of Trochus, and can be divided into two portions, the dorsal portion, consisting entirely of glandular tissue, extending up between the pericardium and the stomach, and the ventral portion, which is lined by a thin membranous wall, forming a kind of urinary chamber (*k. c.*) into which the excreted products of the gland are collected. This urinary chamber is continued on as a thin-walled ureter (*u*) lying on the right side of the mantle-cavity to the right of the rectum, and opening to the exterior by an aperture situated close to the aperture of the left kidney.

In all the species of the sub-genus Gibbula (figs. 39—41) the external aperture of the right kidney is bounded by tumid lips, the borders of which are fringed. This swollen expansion of the terminal portion of the ureter is very conspicuous in females, more especially so during the breeding season. Numerous mucus-secreting cells are present in this enlarged portion.

In T. zizyphinus (figs. 42, 49) and other members of the

sub-genus Calliostoma the terminal portion of the ureter becomes very much enlarged, forming what Perrier terms an ampulla (*amp.*). This enlargement is present only in the female, and the lumen of the ureter is here very small, becoming almost obliterated by the relatively enormous thickness of the walls (fig. 49). The external aperture of the ureter is placed at the termination of this thickening. The walls of the ampulla contain numerous mucus cells, which swell up enormously when they come in contact with water. A similar enlargement of the ureter has been described by Woodward as occurring in the female of Pleurotomaria Beyrichii. It is undoubtedly an accessory to the female genital organs, and from its very glandular nature it seems probable that it is concerned in the secretion of the albuminous material in which the eggs are enveloped prior to their discharge. Though this structure is by no means so highly developed in the members of the sub-genus Gibbula, it is undoubtedly represented by the tumid and fringed lips at the anterior extremity of the ureter.

The presence of an anterior lobe to the right kidney and the accessory genital organ in the female of certain species of Trochus undoubtedly proves the very close affinities of the Trochidæ to Pleurotomaria, in which identically the same structures are present. Also the presence of these two structures in certain species and their almost entire absence in others serve very well as a basis upon which we can definitely separate the species enumerated into the two well-marked sub-genera Calliostoma and Gibbula.

Until quite recently no connection had been traced between the right kidney and the pericardium, and it was thought that the right reno-pericardial canal had been lost. Pelseneer, however, in 1898 (**36**, p. 53), described a right reno-pericardial canal in Trochus cinerarius. My own researches confirm this observation, as I have been able to demonstrate, both by dissection in T. lineatus (fig. 48, *r. p. c.*) and by the examination of serial sections in T. magus (fig. 35, *r. p. c.*), that such a communication does exist. The right reno-

pericardial canal does not open directly into the kidney, but into the genital duct at the point where it debouches into the urinary chamber. In some of the females that were obtained during the breeding season ova were found inside the pericardium, thus demonstrating the existence of a direct communication between the pericardium and either the genital duct or the urinary chamber. Fleure (**16**) has recently described the existence of a right reno-pericardial pore in Haliotis, and mentions the fact that ova were frequently found in the pericardium, having been introduced into that chamber viâ the reno-pericardial channel.

The structure of the glandular portion of the right kidney has been described by Perrier (**37**) as consisting of a sac divided by numerous trabeculæ, these being lined with glandular cells. Haller (**21**) and Pelseneer (**36**, p. 53) regard it rather as a gland composed of a number of acini, the cavities of the acini uniting into principal branches, which lead into the urinary chamber. This, according to my observations, appears to be the true interpretation of the structure of this kidney. The excretory cells (fig. 37) are pear-shaped bodies with very large nuclei and very granular protoplasm, in which are embedded large round granules of a greenish colour, evidently products of excretion. The ciliated cells (fig. 37) lining the main passages of the acini and the urinary chamber are much smaller than the true excretory cells, the protoplasm is not so granular, and they rarely if ever contain any excretory granules.

Genital Organs.—The genital gland (figs. 39, 40, *g. g.*) is in both sexes situated external to the liver, and extends up to the termination of the spire of the visceral mass. A difference of colour in this gland is almost the only character by means of which the male can be distinguished from the female.

In T. lineatus the male gonad is pink, while that of the female is green in colour. In both sexes the genital products are discharged through a genital duct (figs. 35, 36, *g. d.*) into the urinary chamber of the right kidney. This duct was first

correctly described by Pelseneer (**36**, p. 54), who found that it opened into the right reno-pericardial canal. The genital duct, or rather that portion which is common to the right reno-pericardial canal and the genital duct, opens into the right kidney on a small papilla (fig. 36, *g. d.*). From the cavity of the right kidney the genital products are discharged into the mantle-cavity through the ureter. In the male the ureter is quite unmodified, but in the female the terminal portion is enlarged, either as a thick-walled ampulla, as in members of the sub-genus Calliostoma (figs. 43, 49, *amp.*), or as a rosette-shaped enlargement in the members of the sub-genus Gibbula (figs. 39—42).

The Alimentary Canal.—The mouth, situated on the ventral surface of the snout, leads into a thick-walled, muscular, buccal cavity, on the antero-lateral walls of which are placed two chitinous jaws (figs. 12, 13). These jaws are moderately well developed in both T. zizyphinus (fig. 12) and T. granulatus; each jaw being made up of two portions—a large outer plate-like part and an inner smaller structure, the free margin of which is irregular, and fringed with chitinous projections. In T. magus (fig. 13) and the remaining species of Trochus examined the jaws are comparatively small and insignificant, consisting of very thin membranous structures composed of chitinous tesseræ, which are more or less restricted to the free margins; there is no indication of the small inner plate that occurs in T. zizyphinus.

A section through the jaw and its associated parts reveals the fact that each rod-like chitinous tessera is secreted by a single cell (fig. 14). On the outer margin of the jaw there is a thin limiting membrane (*o. m.*) covering the exposed faces of the tesseræ (*t. s.*); the tesseræ are long rod-like bodies closely applied to each other; they present a finely striated appearance, the striæ being arranged in a longitudinal direction. Immediately underlying these and attached to their basal ends are the formative cells (*f. c.*), each tessera being connected to an individual cell. These cells are

elongated bodies, whose protoplasm is finely granular, the granules being arranged in longitudinal striæ; each cell encloses a large oval nucleus.

The formative cells rest upon a clear, thin, structureless basement membrane (*b. m.*), which is in turn succeeded by a layer of muscle-fibres (*m. f.*) with elongated nuclei.

In many of the exotic Trochidæ (e. g. T. niloticus, etc.) jaws are entirely absent.

Closely attached to the body-wall by radiating muscle-fibres is the buccal mass (figs. 39, 40, 44); this is a very muscular structure, and is supported by the large odontophore (*od.*), consisting of two pairs of odontophoral cartilages; the larger and anterior pair serve mainly for the support of the radula, while the smaller basal and posterior pair present concave surfaces upon which the anterior cartilages articulate, and also serve as fixed points for the attachment of the majority of the protractor and retractor muscles of the odontophore.

The radula is extremely long, and is ensheathed in a radula-sac (*r. s.*), which, after emerging from between the anterior pair of odontophoral cartilages, becomes involved in the general torsion of the body, and, though situated ventral to the crop anteriorly, is twisted over the right side, so that the posterior portion eventually comes to lie on the dorsal surface of the crop.

The terminal portion of the radula-sac is bifid in T. lineatus (fig. 40, *r. s.*), T. magus (fig. 39, *r. s.*), and all other species belonging to the sub-genus Gibbula. In T. granulatus and T. zizyphinus there is no trace whatever of this bifurcation.

The radula of Trochus is typically rhipidoglossate. Troschel (**42**) has figured and described the radulæ of numerous species of the Trochidæ.

Amongst the species enumerated in this paper very little difference in radula structure occurs. We can, however, distinguish between two fairly distinct types, represented by T. granulatus and T. zizyphinus on the one hand and

T. magus and the remaining species on the other. In the former (figs. 20, 21) the radula is characterised by the extremely large size of the first or admedian marginal tooth, also by the serrated edges of the cusps of both the central and lateral teeth. In the latter the cusps of the central and lateral teeth are devoid of serrations, but the lateral teeth are notched on their distal margins, and the central tooth has notches on both sides of the basal portion of the cusp (figs. 15, 18, 19, 28, 29). The first marginal tooth of these species is also of considerable size, but not so large relatively as in T. granulatus or T. zizyphinus. In T. lineatus (fig. 19), on the contrary, the first marginal tooth differs in no way from the succeeding ones.

In each transverse row of teeth of the radula of Trochus the following clearly defined regions can be distinguished. An unpaired median or rachidian tooth, bordered on either side by five lateral teeth, succeeding which is an indefinite number of marginal teeth or uncini. We can represent the dentition of the radula by a formula as follows:

$$\infty \; 5 \; | \; 5 \; \infty$$

The marginal teeth vary considerably in shape and size, those nearer the central tooth being stouter and shorter than those more remote. The majority of the marginal teeth or uncini are hooked (figs. 16, 17, 22—24). The teeth situated some distance from the centre become slender and elongate (figs. 24, 25). In T. zizyphinus and T. granulatus these distal teeth are characterised by the deep serrations on the margins. In teeth still more remote these serrations (fig. 26) become still deeper, and give a brush-like appearance to the teeth, though they cannot be compared to the brush-teeth of Pleurotomaria (**45**, p. 250, figs. 46—52).

At the extreme distal end of the marginal teeth some nine or ten specialised teeth are situated. These are flattened, and present neither serrations nor notches on the margins. They

are spread out in a fan-like manner, and constitute the flabelliform teeth (fig. 27).

It will be seen on examination of figs. 28 and 29 that the radulæ of T. striatus and T. exasperatus approximate more nearly to the Gibbula than to the Calliostoma type, in that the cusps of the central and lateral teeth are unserrated, but bear on their distal margins very distinct notches, such as are present in T. magus.

It is almost impossible to compare the radula of Trochus with that of Pleurotomaria, as in the latter we find no trace of the clearly marked regions which the radula of Trochus presents. The radula of Pleurotomaria is also obviously specialised in the possession of such extremely modified structures as the brush and lamellate teeth. A peculiar feature of the Pleurotomarian radula is the presence of a series of accessory basal plates, situated underneath, and alternating with the bases of the uncinate teeth (Woodward, **45**, p. 252, fig. 32). A similar series of basal plates is present in the radula of Trochus, occupying a corresponding position, viz. at the base of the uncinate or marginal teeth.

The salivary glands are slightly different in the two sub-genera Gibbula and Calliostoma. In the former they are small rod-like bodies (figs. 39, 40, *sl. g.*) lying on the dorso-lateral surfaces of the anterior portion of the crop, and opening into the buccal mass slightly in front of the cerebral commissure. In T. zizyphinus (fig. 44, *sl. g.*) and other species of Calliostoma the salivary glands are larger and racemose. The duct opens into the buccal cavity immediately over the anterior end of the odontophore.

The Crop.—The anterior portion of the alimentary canal is enlarged to form the crop (fig. 39, *cr.*); upon the dorsal surface a rod-like area can be distinguished, which curves over from the mid-line towards the left side, eventually becoming ventral in position.

Communicating with the crop are two lateral diverticula, viz. the right and left œsophageal pouches, the former being the larger.

Evidence of torsion having affected the alimentary canal is furnished by the displaced condition of the posterior portion of the radula-sac (vide p. 50) and by the rotation of the right œsophageal pouch to the left side, and vice versâ (**38**, p. 392). Torsion of the crop and its associated structures has been described by Woodward (**45**, p. 236) in Pleurotomaria, and in Turbo and other genera by Amadrut (**1**)

Just beyond the point at which the radula-sac crosses over the dorsal surface of the crop this latter becomes much smaller and thicker walled, and may be regarded as the œsophagus (figs. 40, 45, *œ.*); it passes backwards and ultimately opens into the posterior portion of the stomach.

The stomach (figs. 39, 40, 45, *st.*) is situated underneath and behind the right kidney, and is a large sac divided into an œsophageal or posterior and an intestinal or anterior chamber. From the posterior region of the stomach there arises a large spiral cæcum (*sp. c.*), a structure characteristic of the majority of the Diotocardia.

There is a slight difference in the shape of the stomachs in the members of the sub-genus Gibbula and those of the sub-genus Calliostoma. In the latter this organ is more or less **U**-shaped, and the spiral cæcum arises at the bend of the **U**, near the confluence of the œsophageal and intestinal chambers; the intestine leads directly out of the latter, and does not coil on itself in the manner in which it loops in T. lineatus (fig. 45) and other species of the sub-genus Gibbula.

In Calliostoma the spiral cæcum consists of many turns, and the apex of the spire can be distinctly recognised on the outer surface of the visceral mass. In Gibbula, on the contrary, the spiral cæcum consists of few turns, and the apex of the spire is deeply buried in the substance of the liver, only the basal coil being visible on the exterior.

When the interior of the stomach is examined (fig. 45) two conspicuous folds, arising in the vicinity of the œsophageal aperture, are plainly visible. These folds are continued up to and throughout the whole length of the spiral cæcum, en-

closing between them a cæcal groove (*cæ. g*). Within this groove, and situated in close proximity to the aperture of the œsophagus, the larger of the two bile-ducts opens (*b. d*). It may be regarded as a point of considerable interest that in all Gasteropods in which a spiral cæcum is present, and also in many of the Cephalopoda in which a cæcal diverticulum of the stomach exists, whether spiral or otherwise, there is always this relationship between the aperture of the bile-duct and the folds, or rather, the cæcal groove bounded by the folds leading into the spiral cæcum or stomachic diverticulum. This correlation of structure exists in such archaic forms as Pleurotomaria, Nautilus, and Spirula (Moore, 30), and is undoubtedly indicative of the homology of the spiral cæcum of the Gasteropods and the cæcal diverticulum of the Cephalopod stomach.

The stomach of Trochus is lined with a thin membrane of a chitinous nature (fig. 46, *cut.*). This cuticle is a product of secretion of the epithelium (*g. ep.*) of which the wall of the stomach is mainly constituted; this epithelial layer is composed of very elongate columnar cells with large nuclei. The upper portion of these cells, viz. that part immediately underlying the cuticle, presents a finely striated appearance. Between this striated border and the nucleus the protoplasm of the cells is very granular, owing to the presence of numerous small bodies of a greenish colour; these are probably of the nature of enterochlorophyll, and comparable to the granules of enterochlorophyll described by McMunn as present in the epithelial cells lining the stomach of Patella.[1]

Subjacent to the gastric epithelium is a thin layer of muscle-fibres with elongate nuclei, and this layer is further surrounded by a loose connective tissue, many of the cells of which contain large granules analogous to those found in the excretory cells of the right kidney. These (fig. 46) are the

[1] C. A. MacMunn, "On the Gastric Gland of Mollusca and Decapod Crustacea; its Structure and Function" ('Phil. Trans. Roy. Soc. Lond.,' vol. cxciii, B. 11, 1900).

plasmatic cells of Brock (9), and appear to be of common occurrence in the connective tissue of Gasteropods.

The intestine either leads directly out of the anterior or intestinal chamber of the stomach without becoming folded upon itself as in T. zizyphinus, or it recurves and crosses over the stomach as in T. lineatus (figs. 40, 45, *int.*); becoming folded upon itself several times, it then runs forward to about the level of the terminal portion of the radula sac, where, bending on itself to form a U-shaped loop, it retraces its course towards the posterior end of the body, and on reaching the level of the pericardium curves dorsally and horizontally, entering the pericardium and penetrating the ventricle. After emerging from the pericardium it again curves, and entering the mantle-cavity runs along the roof of that structure towards the anterior end of the body, debouching into the mantle-cavity by the anus, which is situated near the middle line. The terminal portion of the rectum (*r.*) is enveloped by the hypobranchial gland (*m. g.*).

The Vascular System.—The heart (figs. 39, 47) is enclosed within a large pericardium, which is situated at the distal end of the mantle-cavity, abuts on the left kidney, and is bounded on its posterior border by the right kidney and stomach. The ventricle (*v.*) is traversed by the rectum and is very muscular. It is situated nearly transversely, passing from right to left of the body; on the left side the ventricle is enlarged into a bulbous structure, the aortic bulb, from which arise two large arteries, the posterior and anterior aortæ. Communicating with the ventricle are two thin-walled auricles; of these the left (*l. au.*) is the larger, and is situated in the anterior portion of the pericardium; the right auricle (*r. au.*) is situated in the posterior region of the pericardium, and, though of smaller calibre than the left, is much longer. The walls of both right and left auricles are very thin, and are produced into numerous fringe-like processes which, when examined microscopically, are seen to be clothed with numerous large epithelial cells (fig. 38), each containing a large round nucleus and protoplasm having

a granular appearance. These cells are manifestly glandular, and present a very striking resemblance to the excretory cells of the right kidney; they constitute the so-called pericardial gland, and according to Grobben [1] and Perrier (**37**, p. 127), the products of excretion are conveyed out of the pericardium to the exterior through the left reno-pericardial canal and papillary sac.

The posterior aorta (figs. 39, 47, *p. ao.*) arises from the aortic bulb, crosses over the right kidney and stomach, giving off branches to the latter; it then curves under this organ, follows the inside of the visceral spire to its apex, and distributes branches to both liver and gonad.

The anterior aorta (*a. ao.*), which also arises from the aortic bulb, is situated on the left side of the body between the body-wall and the ascending portion of the intestine. It follows the course of the intestine for a considerable distance, furnishing it with several branches, crosses to the right, passing over the crop, and penetrates between the crop and radula-sac; it supplies the buccal mass with vessels, and then recurves to form a sinus situated above the ventral nerve-cords; from this the blood penetrates into the lacunæ of the foot.

The venous system is chiefly lacunar, sinuses being conspicuous in the foot, especially in the glandular portion on the dorsal surface. The blood returning from the posterior region of the visceral mass traverses the right kidney by numerous sinuses; these are collected into a large vessel, the efferent renal vein (fig. 48, *e. r. v.*), which passes into the mantle-cavity, where it unites with a vessel bringing blood from the sinuses of the anterior portion of the body; the vein formed by the union of these vessels crosses over the rectum, and, emerging from between the apertures of the right and left kidneys, traverses the mantle from right to left as the transverse pallial vein (figs. 39—43, *t. p. v.*); it receives

[1] Grobben, C., "Die Pericardialdrüse der Lamellibranchiaten (ein Beitrag zur Kentniss der Anatomie dieser Molluskenclasse)," 'Arb. zool. Inst. Wien.,' Bd. vii, 1888.

vessels bringing blood from the lacunæ of the anterior portion of the mantle and the perirectal sinus. This vein then runs along the branchial support, distributing blood to the lamellæ of the gill, constituting in fact the afferent branchial vein. Part of the blood conveyed by the transverse pallial vein is distributed directly to the left kidney by two sinuses (fig. 42) arising from that vein as it crosses over the rectum and emerges between the renal apertures. These sinuses follow the right and left borders of the papillary sac, and communicate with the lacunæ of that organ. The blood, after passing through the lacunæ of the papillary sac, is collected into a small vessel which communicates directly with the left auricle. After aëration, the venous blood distributed to the gill is collected into a large efferent branchial vein (figs. 39—43, *e. b. v.*), which runs along the base of the gill and conveys the arterialised blood to the left auricle.

The right auricle also communicates with the lacunæ of the papillary sac, receiving some of the venous blood passing through that organ. In consequence of the suppression of the right gill there is no functional efferent branchial vessel communicating with the right auricle, though it is possible that a very small vessel which runs on the mantle-wall underneath the rectum and communicates with the right auricle may, according to Thiele (**41**), represent a vestige of the right efferent branchial vein.

Nervous System.—The nervous system of the Trochidæ presents no differences of importance in any of the species so far examined. Such forms as T. striatus, T. tumidus, etc., being far too small for satisfactory results to be obtained by dissection, were embedded in paraffin wax and cut into serial sections, and from an examination of these sections the main features of their anatomy were subsequently made out, the nervous system being reconstructed by the method of building up in wax.

The distribution of nerve-cells is of particular interest. In Pleurotomaria there is a very general distribution of nerve-cells throughout a greater part of the nervous system (Wood-

ward, **45**, p. 240), occasionally on the nerves themselves as well as on the commissures and connectives. In this genus there is scarcely any aggregation of nerve-cells into ganglia, the only indication of definite nerve-centres being the points of origin of the various characteristic nerves.

In Trochus, however, the nervous system is more highly developed, there being definite ganglia in which a concentration of nerve-cells has taken place, and moreover, though nerve-cells may occasionally occur on the various connectives, they are practically absent along the commissures, and are thus much more restricted with regard to their localisation and distribution than is the case in Pleurotomaria.

The cerebral ganglia (figs. 30, 40, 44, *cb. g.*) are situated on the sides of the anterior portion of the buccal mass, and are united with each other by a long cerebral commissure (*cb. c.*). Nerves are given off from these centres to the snout, the cephalic lappets, the tentacles, and the eyes, the branches innervating these two latter structures being quite distinct, and not, as occurs in Pleurotomaria, arising from a common root. From the ventral portion of the cerebral ganglia a rather broad band is given off, from which two important nerves arise; one of these, at first comparatively large, but eventually becoming thin and delicate, passes laterally and ventrally under the buccal mass, uniting with its fellow of the other side, and forming the labial commissure (figs. 30, 44, *l. c.*). The other nerve which arises from the enlarged portion of the labial commissure is the buccal or stomatogastric nerve (figs. 30, 44). It curves upwards over the odontophore and penetrates between this structure and the dorsally situated œsophagus, where it enlarges into the buccal ganglion (*b. g.*). The buccal commissure which unites the ganglia of either side is as well supplied with nerve-cells as the ganglia themselves, and it is only by the slight enlargement of the commissure into two masses that we can speak of definite buccal ganglia. Several nerves are given off both from the ganglionic enlargements and the commissure;

these are distributed to the crop, salivary glands, and the odontophore.

This peculiar method of origin of the stomatogastric nerves in Trochus, in arising from the same root as the labial commissure, finds its parallel not only in Pleurotomaria (Woodward, **45**, p. 242), but also in Patella and Chiton (Pelseneer, **36**, p. 48). The extreme fineness of the connectives uniting the buccal ganglia to the cerebrals, and the fact that they are only indirectly connected with the latter, arising in reality in common with the labial commissure, is in all probability the reason which led Béla Haller (**19**, p. 26, pl. ii, fig. 3) to overlook the true point of origin of these nerves, and to suppose that they originated from the sub-œsophageal mass.

From the posterior border of each cerebral ganglion two long connectives, the cerebro-pedal (*cb. p.*), and the cerebro-pleural (*cb. pl.*) arise, the latter being the larger of the two. These cords pass backwards over the odontophore and penetrate the floor of the body-cavity, where they unite with the large ganglionic mass, representing the pleural and pedal ganglia.

The pleural ganglia (*pl. g.*) in Trochus are perfectly distinct structures, and are situated at the anterior extremity of the ventral or pedal nerve-cords (figs. 30, 40, *pl. g.*) as two projecting horns immediately in front of the anterior commissure which unites the pedal cords. The close approximation of the pleural and pedal ganglia is undoubtedly a specialised condition, and is in all probability due to the shortening of the pleuro-pedal connective, which in Trochus has become almost entirely obliterated, the basal portion of the pleural being fused to the anterior portion of the large ventral pedal nerve-cords. Such a condition, though unusual in Prosobranchiate Gasteropods, is not unique, being met with in Cyclophorus and also in Ampullaria.

From the pleural ganglia are given off right and left pallial nerves (figs. 30, 39, *pa. n.*, *pa. n'.*). These branch shortly after entering the mantle, the anterior nerves being distributed

to the anterior thickened margin of the mantle, where they eventually unite with one another, forming a circumpallial anastomosis (Pelseneer, **36**, p. 50). The posterior branch of the pallial nerve is distributed to the posterior portion of the mantle which ensheathes the columella muscle. In addition to the pallial nerve a collumella nerve is given off from the pleural ganglion.

Visceral Commissure.—The right or supra-intestinal branch (fig. 30, *sp. int.*) of the visceral loop arises from the right pleural ganglion slightly in front of the pallial nerve of this side. It passes upwards over the odontophore and through a fold in the dorsal wall of the crop to the left side of the body, where it penetrates the body-wall. Here it gives origin to two nerves, one going to the large branchial ganglion (*br. g.*) which is situated at the base of the gill, the other nerve (*d.*) running to and anastomosing with the left pallial nerve, thus presenting a condition of dialyneury on the left side of the body. At the point of origin of these two nerves there is a slight enlargement and concentration of nerve-cells, and we can consequently look upon this centre as representing the supra-intestinal ganglion, though it is by no means so large or so clearly defined as delineated by Pelseneer (**36**, pl. xvii, fig. 148). The branchial ganglion innervates both the gill and the osphradium. The supra-intestinal branch of the visceral commissure then continues its course along the left side of the mantle-cavity, situated in the angle between the body-wall and the gill, it runs parallel to the latter structure until it reaches the level of the papillary sac, where it crosses the body from left to right, passing above the œsophagus and intestine, and terminating in the abdominal ganglion (*ab. g.*) which is situated under the epithelium of the floor of the mantle-cavity.

The subintestinal branch (fig. 30, *sub. int.*) of the visceral loop arises from the left pleural ganglion by a trunk common to both this nerve and the left pallial nerve; it then passes underneath the œsophagus and radula-sac, and continues its course on the right side of the body between the œsophagus

and the columella muscle until it reaches the aforementioned abdominal ganglion. There is no trace of a subintestinal ganglion, and neither by the method of dissection nor by the examination of serial sections have I been able to make out any trace of an anastomosis between the subintestinal nerve and the right pallial nerve, though such a connection has been indicated by Bouvier (8, p. 171, fig. D).

The common origin of the subintestinal branch of the visceral commissure with the left pallial nerve does not appear to have any special morphological significance, as in one specimen of T. cinerarius, the nervous system of which was modelled in wax from serial sections, exactly the reverse condition obtained, the supra-intestinal nerve and the right pallial nerve having a common origin from the pleural ganglion, the subintestinal branch arising in front of the left pallial nerve.

The abdominal ganglion (*ab. g.*) gives origin to three important nerves. One arising anteriorly is distributed to the rectum, a median large branch, the visceral nerve (*v. n.*), runs along the inside of the visceral spire and innervates the stomach, liver, and genital gland, while the third nerve is distributed to the right kidney and heart.

The visceral loop in Trochus is typically streptoneurous.

The ventral or pedal nerve-cords (figs. 30, 40, *pd. c.*) are paired structures running in the muscular mass of the foot throughout its entire length. On their outer lateral surfaces they are superficially divided into halves by a longitudinal groove (fig. 40). At the anterior end of the foot these cords approximate one another closely, and are united by a thick anterior pedal commissure. As they proceed through the muscle of the foot they diverge slightly, being furthest apart at their middle portion, and begin to converge again as the posterior end of the foot is reached.

In addition to the thick anterior pedal commissure there are numerous thin transverse commissures joining the pedal cords together, and giving to them their characteristic scalariform appearance. Ganglion-cells are distributed evenly

on the periphery of the pedal cords throughout their whole length, but are not concentrated into any particular place which might be termed a pedal ganglion. There is an entire absence of nerve-cells on the transverse commissures.

Numerous nerves are given off from the pedal cords; from their external lateral surfaces nerves are distributed to the epipodia and lateral portions of the foot, while on the ventral surface large nerves originate, and are distributed to the ventral portion of the foot.

With respect to the composition of these ventral or pedal nerve-cords of Trochus and the Diotocardia generally, there is a considerable amount of diversity of opinion, and this has led to a somewhat lengthy discussion between the supporters of two theories that exist at present.

One of the views held concerning the composition of the pedal nerve-cords is to the effect that they are of a double nature, consisting of both pleural and pedal elements; while the other view regards the nerve-cords as being purely pedal.

The chief exponent of the former view is Lacaze Duthiers, who bases his opinion upon anatomical grounds and relationship of parts. During his investigation on the nervous system of Haliotis (**26**, p. 272) he came to this conclusion, and at the same time promulgated the theory that the epipodium was a pallial structure. Later on he extended his observations to the Trochidæ (**27**), and found the same condition existing in the pedal cords of this family. In the longitudinal cords of both Haliotis and Trochus, and also as has recently been demonstrated in Pleurotomaria, there is on the outer surface an external groove running along them to their extremities, and dividing them superficially into an upper and lower half. Moreover in certain of the Trochidæ there is a still further distinction in the fact that the upper half is white in colour, while the lower part is yellow. Lacaze Duthiers regards the upper portion of the cords as pleural in nature and the lower part as pedal. The nerves given off to the epipodium are, according to this view, conceived as

arising wholly from that portion of the ventral nerve-cord which is situated above the longitudinal groove, and are therefore pleural, while the nerves distributed to the foot arise from the lower half of the cord, and hence are exclusively pedal; the epipodium being consequently a pallial structure.

Spengel (**39**, pp. 343, 344), Haller (**19**, pp. 3, 22), Thiele (**40**), and Pelseneer (**31—35**) deny this double nature of the pedal cords, and can see no apparent trace of any morphological separation into halves. They base their opinion on histological grounds, and find from the examination of sections that, though a conspicuous longitudinal groove is present on the outer side of each cord, there is no trace of histological differentiation between the halves of the cords separated by the groove, and moreover, that microscopical examination with the highest powers fails to reveal the presence of any connective tissue separating them. Lacaze Duthiers (**29**) agrees with Spengel as to the entire absence of any connective tissue sheath between the halves of the cords, but he asserts that this does not indicate the absence of any separation, that the separation is not necessarily a histological one, and that there is most decidedly a physiological differentiation of the nerve-cords; he cites in confirmation of his view the fact that in the majority of Gasteropods (Patella, for example) the auditory nerve, which runs from the cerebral ganglion to the otocyst, is indistinguishably fused with the cerebro-pleural connective, and that there is no connective-tissue sheath separating the auditory nerve from the connective. There is, however, a physiological separation between the two nerves.

This view is held by other investigators. Wegmann (**44**) considers that the epipodium of Haliotis is a pallial structure, and that the nerve innervating it is pleural in origin, as it arises from that portion of the pleuro-pedal (?) or ventral nerve-cord situated above the longitudinal groove. He has found that during dissection the pleuro-pedal cord is apt to break, the rupture occasionally taking place in such a manner as to separate the pleural from the pedal half

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moreover, the epipodial nerve has come away intact with the pleural portion of the cord, while those nerves distributed to the foot have remained on the pedal half.

Boutan also supports the theory of the double nature of the pedal cord from his investigations on the anatomy of Fissurella (3) and Parmophorous (4). In the latter genus he distinguishes three kinds of nerves given off from the ventral nerve-cord: (1) from the lower surface, nerves which go exclusively to the foot; (2) laterally, nerves distributed to the collarette, i. e. the epipodium or inferior mantle; (3) between these latter, nerves which go directly to the mantle; thus both pedal and pleural nerves are given off from the lower and upper halves respectively of the ventral nerve-cord.

Bouvier and Fischer (8) also regard these nerve-cords as consisting of pleural and pedal halves and the epipodium as a pallial structure; they, however, consider that many of the nerves given off from these cords contain fibres from both pleural and pedal halves, that these nerves in fact consist of mixed fibres.

If, however, the ventral nerve-cords are purely pedal, as Spengel and others maintain, it is obvious that the epipodium, being innervated from a pedal centre, must be regarded as an outgrowth of the foot, having no connection whatever with the mantle.

Arguments in favour of this view are based upon histological investigations. Haller (20) finds that in Turbo nerve-fibres pass from the upper to the lower portion of the ventral nerve-cord. Again, Woodward (45) finds the same condition obtaining in Pleurotomaria. Pelseneer, who has always maintained that the epipodium is a pedal structure, and that the ventral nerve-cords are entirely pedal, has recently (36, p. 49) shown that the epipodial nerves receive fibres from both upper and lower halves of the nerve-cords. From the examination of numerous serial sections, both transverse and longitudinal, of various species of Trochus I have been able to confirm this observation of Pelseneer's, and find that the nerves going to the epipodium have a double origin

(fig. 31), receiving fibres from both upper and lower halves of the cords. This would necessarily indicate that the epipodial nerve is constituted in part, at any rate, of pedal fibres; and if we consider with Lacaze-Duthiers, Bouvier, etc., that the upper part of the ventral nerve-cord is pleural in nature, then the epipodium has a mixed innervation, its nerve being composed of both pleural and pedal fibres. But the examination of other sections has revealed that this mixing of fibres is not confined exclusively to the epipodial nerves. The transverse commissures between the pedal cords are themselves composed of fibres from both halves of the cord (fig. 32). These commissures apparently connect only the lower halves of the cords, and it is only in sections that we can see that they originate from the upper as well as the lower halves of the cords. Again, fibres from the top portion of the cord may be distributed to definitely pedal nerves. Woodward has described such a condition as occurring in the large latero-ventral pedal nerves of Pleurotomaria, in which fibres are received from both upper and lower portions of the cord, these often forming a conspicuous double root to the nerves. The transverse commissures connecting the pedal cords of Pleurotomaria are, as in Trochus, composed of nerve-fibres from both halves of the cords.

A conclusive proof of the purely pedal nature of the ventral nerve-cords is in my opinion furnished by the transverse section (fig. 33) of the foot of Trochus. Here we have a large nerve given off from the ventral surface of the pedal cord and distributed to the sole of the foot; this receives fibres chiefly from the lower half, but in addition it has a bundle of fibres running to it from the very top portion of the ventral nerve-cord, and these fibres are partially separated from the lower half of the cord by a mass of ganglion-cells. We have thus a nerve supplying only the foot, consisting of fibres from both portions of the cord, and unless we regard the ventral cords as being purely pedal in composition we have the anomalous condition of an undoubtedly pedal nerve consisting of both pedal and

pleural fibres. It seems much more rational to regard these structures as entirely pedal, and consequently the whole of the ventral nerve-cords as purely pedal in composition; in this case the epipodium must be looked upon as an outgrowth of the foot, supplied by pedal nerves, and we can only regard as pleural centres or ganglia the two ganglionated horns which lie dorsal to the pedal centres, and from which are given off the visceral commissures and the pallial nerves. In Pleurotomaria the pleural centres are not so well defined as in Trochus; the visceral loop arises from the cerebro-pleural connective, no definite concentration of nerve-cells into ganglia having occurred. Here we must look upon that part of the connective between the cerebral centre and the pedal cords from which the visceral loop and pallial nerves are given off as alone representing the pleural centres, no pleural elements whatever entering into the composition of the ventral nerve-cords.

In Trochus the more definite concentration of nerve-cells into a pleural ganglion, and the shortening of the pleuro-pedal connective, causing the close proximity of the pleural to the pedal centre, constitute the main differences between the nervous system of this genus and that of Pleurotomaria.

The Sense Organs.—The eye consists of a pigmented optic cup communicating with the exterior by means of a small circular aperture in the cornea. Filling the interior of this cup is a large spherical vitreous body, the crystalline lens.

The histology of the eye has been investigated by Hilger (22).

The otocysts (fig. 30, *ot.*) are large sac-like bodies lying on the upper surface of the anterior extremity of the pedal nerve-cords. The auditory nerve (*ot.n.*) passes from the otocyst over the upper surface of the pedal ganglion and runs to the cerebro-pleural connective, which it accompanies to the cerebral ganglion. At the point where the otocyst nerve communicates with the auditory sac a small diverticulum of the sac enters,

and runs some little distance into the nerve. This diverticulum, though destitute of specialised sensory cells, contains several of the numerous otoconia that are present in the auditory sac.

Lacaze Duthiers, in his memoir on the otocysts of Molluscs (**27**), has described a somewhat similar condition in Patella.

The osphradium (figs. 41—43, *os.*) is a small patch of specialised sensory epithelium of a yellowish colour situated under the branchial ganglion, and extending for a short distance along that portion of the gill-base which lies free in the mantle-cavity. Bernard (**2**, pp. 167—173) has given a detailed account of the histological structure of the osphradium.

Other sense-organs are the cephalic and epipodial tentacles, which are undoubtedly tactile. The epipodial papillæ have probably a similar function.

Sensory cells occur in the buccal cavity of Trochus, similar to those described by Haller (**19**, pl. vii, fig. 28) as occurring in the buccal cavity of Fissurella, and may be gustatory in function.

In addition a peculiar series of sensory organs, first mentioned by Thiele (**41**), is found occurring in the mantle-cavity on the right side, in the angle between the mantle and body-wall.

Conclusions.—It will be seen from the foregoing account that the various species of Trochus examined present very few anatomical differences; it is, however, possible to distinguish between two slightly diverse types of organisation, the characters of which are sufficient to constitute different sub-genera. Retaining the existing nomenclature, we have the one sub-genus Calliostoma, in which the shell is pyramidal, and into which the following species can be placed:—T. zizyphinus, T. granulatus, T. striatus, T. exasperatus, and T. Montagui. In another sub-genus, Gibbula, we can include the remaining forms, viz. T. magus, T. cinerarius, T. umbilicatus, T. tumidus, and T. lineatus. The sub-genus Trochocochlea, in which this latter species

has previously been placed by conchologists, cannot be retained, as the internal organisation of this species, and also that of T. turbinatus (Born), as described by Robert (38), another species previously included in the sub-section Trochocochlea, is almost identical with the anatomical structure of T. magus or other species of Gibbula.

As I was unable to obtain any specimens of species belonging to the so-called sub-genus Margarita (Leach), I cannot say whether sufficient anatomical differences occur to warrant the existence of this separate sub-genus.

So far, then, anatomical investigations have revealed such striking similarity of structure as to necessitate the reduction of sub-genera amongst British Trochidæ, and it is highly probable that an anatomical examination of exotic species will still further considerably reduce the very numerous sub-genera into which these have been classified.

Although both T. zizyphinus and T. granulatus differ in many ways from T. magus and other species of Gibbula, yet the smaller species, T. striatus and T. exasperatus, though they have been included in the sub-genus Calliostoma, agree in some respects more closely with T. magus and its allies than with T. zizyphinus. This is chiefly in respect to their external characters; both of these small forms possess epipodial papillæ and an appendix on the right ocular peduncle, while these structures are absent in T. zizyphinus. Moreover the glandular structure on the dorsal surface of the foot more nearly resembles that seen in T. magus. In respect to the structure of the radula of these species, the condition is an approximation to the Gibbula rather than the Calliostoma type. On the other hand, the presence of a transverse notch on the anterior margin of the foot, and also the enlargement of the terminal portion of the ureter into an ampulla, together with the arrangement of the alimentary canal and spiral cæcum, tend to show their relationship with T. zizyphinus and T. granulatus, and as their shell is pyramidal in shape, it seems necessary to include them in the sub-genus Calliostoma.

The remarkable resemblance of the internal organisation of the Trochidæ, more especially of the species of Calliostoma, to that of Pleurotomaria is of considerable interest as exemplifying the very close relationship which exists between these genera. There is very great similarity existing between the digestive, excretory, circulatory, and nervous systems of these two types. Undoubtedly the nervous system of the Trochidæ is much more specialised than that of Pleurotomaria; there is a greater tendency to the concentration of nerve-cells into definite ganglia, and the close approximation of the pleural ganglia to the pedal ganglia is without doubt a specialisation, the most usual condition in Gasteropods being the approximation of the pleurals to the cerebrals. The suppression of the right gill in the Trochidæ is of little importance when we consider that in Pleurotomaria the right gill begins to show a tendency towards suppression, since it is smaller in size than the left gill. That one gill has been entirely suppressed in Trochus, but that it undoubtedly existed in some ancestral form, is shown by the presence of a vestigial right afferent branchial vein which communicates with the right auricle.

The relationship of the two kidneys in the Diotocardia and the homology of the single kidney of the Monotocardia with either one or other of these has led to considerable discussion, many zoologists maintaining that the single Monotocardian kidney is the homologue of the left kidney or papillary sac of the Diotocardia, while others seek to homologise the Monotocardian kidney with the right one of the Diotocardia. The former view is the more generally accepted, and is based on the relative positions of the kidney and its aperture with respect to the rectum, receiving additional support from the presence in the Diotocardia (Trochus) of a reno-pericardial canal placing the left kidney in communication with the pericardium, and the supposed absence of a similar structure between the right kidney and the pericardium. Further, von Erlanger's researches on the embryology of Paludina (**13**) tend to give support to this view. He

asserts that in addition to the functional kidney which is situated to the right of the anus before torsion there is present a rudiment of the actual right kidney lying to the left of the anus before torsion.

This observation, however, as Woodward remarks (**45**, p. 260), loses its value when we consider that this so-called rudiment of a right kidney is only apparent as a slight outgrowth of the pericardium which quickly loses its identity without ever showing any indication of the character of a true kidney.

On the other hand, Perrier (**37**) seeks to homologise the single kidney of the Monotocardia with both kidneys of the Diotocardia, comparing the true excretory portion with the right kidney and the nephridial gland with the left kidney or papillary sac. Thus he considers that the two distinct kidneys of the Diotocardia have been united to form the single excretory organ of the Monotocardia.

Woodward also supports this view, and, mentioning that through suppression of the right gill the two kidneys of the azygobranchiate Diotocardia approach each other very closely, he suggests that in early Monotocardia a connection between these two kidneys was formed, thus enabling the excretory products of the right kidney to pass through the left kidney and so to the exterior, while the right kidney-duct, serving for the transmission of the genital products, would eventually become completely separated from the kidney and function entirely as a genital duct, the glandular portion of the papillary sac then degenerating and remaining only as the nephridial or renal gland of the Monotocardia.

Haller (**21**) also maintained the view that the kidney of the Monotocardia was the homologue of the right kidney of the Diotocardia, and in Turbo described the presence of a connection between the right and left kidneys (**21**, figs. 26, 28). This observation is, however, erroneous. In Ampullaria Bouvier (**7**) has described the presence of two kidneys which are in communication with one another, one of them corresponding to the right and the other to the left kidney

of Trochus, and having similar functions and relationships. Burne (**10**) has recently shown that a reno-pericardial canal is present in Ampullaria.

One of the chief objections to regarding the Monotocardian kidney as homologous to the right kidney of the Diotocardia was the supposed absence of any communication between this kidney and the pericardium. This objection has, however, been removed, for Pelseneer (**36**) has shown that a right reno-pericardial canal does exist in Trochus. I have been able to confirm his observation.

In Fissurella, though this is undoubtedly a specialised form, the only reno-pericardial canal present is between the right kidney and the pericardium, and this right kidney is larger and of more functional importance than the left. Again, in Patella there are reno-pericardial canals between the pericardium and both kidneys, though with regard to this genus there has been considerable diversity of opinion, some observers maintaining the presence of a right reno-pericardial canal only, others a left; while v. Erlanger (**14**) denies the existence of any canal whatever.

Cunningham (**12**) was the first to describe the presence of two canals, and lately Goodrich (**18**) has confirmed this observation by means of the examination of serial sections, and still more recently I have been sufficiently fortunate to obtain exactly the same results as Goodrich, also by means of serial sections through the pericardium and kidneys.

In Haliotis the left kidney is relatively very small, and, according to Perrier (**37**), Wegmann (**44**), and v. Erlanger (**14**), it is this kidney alone which communicates with the pericardium. In a recent paper on the kidneys of Haliotis Fleure (**16**) finds that a reno-pericardial canal exists between the right kidney and the pericardium, but denies the existence of a left reno-pericardial canal.

With regard to Pleurotomaria, Woodward (**45**) has described a left reno-pericardial canal only. I have examined his preparations of the kidneys and pericardium, and failed to find any communication between the right kidney and

the pericardium, though the pericardium at the point where a canal might possibly have existed was torn, rendering accurate observation impossible.

We see, therefore, that in the majority, if not all, of the Diotocardia a communication exists not only between the left kidney and the pericardium, but also between the right kidney and that structure, while in some cases only the right canal persists. This is undoubtedly a point very much in favour of regarding the right kidney of the Diotocardia as giving rise in part, if not wholly, to the single kidney of the Monotocardia.

When we come to consider the total difference in function between the right kidney and the left or papillary sac of such forms as Trochus, Haliotis, and Pleurotomaria, it seems much more rational to suppose the kidney of the Monotocardia to have been derived principally from the right kidney of the Diotocardia, for the function of these organs is the same in the two groups—since they are the true excretory organs, whereas the left kidney or papillary sac of Trochus and its allies has an entirely different function. It is more of the nature of a lymphatic gland, waste products being removed from the blood traversing it by a process of phagocytosis (Pelseneer, **35**).

The nephridial gland of the Monotocardia possesses similar functions, and so, from a physiological point of view, can more easily be homologised with the papillary sac of Trochus.

Von Erlanger (**14**), in maintaining the homology of the Monotocardian kidney to the left kidney of the Diotocardia, seeks to homologise the nephridial gland of the former with the right kidney of the latter, but as this necessitates a complete inversion of the functions of these organs, it to my mind seems much more difficult of conception than to accept Perrier's view.

Bibliography.

1. Amadrut.—"La partie antérieure du tube digestif et la torsion chez les Mollusques Gastéropodes," 'Ann. Sc. Nat. Zool.,' 8e sér., t. vii, 1898.
2. Bernard, F.—"Recherches sur les organes palléaux des Gastéropodes Prosobranches," 'Ann. Sc. Nat. Zool.,' 7e sér., t. ix, 1890.
3. Boutan.—"Recherches sur l'anatomie et le développement de la Fissurelle," 'Arch. Zool. expér.,' 2e sér., t. iii, bis, 1886.
4. Boutan.—"Le système nerveux du Parmophorous (Scutus) dans ses rapports avec le manteau, la collerette (manteau inférieur), et le pied," 'Revue biologique du Nord,' t. ii, 1890.
5. Boutan.—Le système nerveux du Parmophorous Australis," 'Arch. Zool. expér.,' 2e sér., t. viii, 1890 (Notes et revue,' p. xliv).
6. Bouvier, E. L.—"Système nerveux, morphologie générale, et classification des Gastéropodes Prosobranches," 'Ann. Sci. Nat. Zool.,' 7e sér., t. iii, 1887, pp. 30—46, and pp. 348 and 481.
7. Bouvier, E. L.—"Études sur l'organisation des Ampullaires," 'Mem. Soc. Philom.,' 1888.
8. Bouvier, E. L., et Fischer, H.—"Étude monographique des Pleurotomaires Actuels," 'Arch. Zool. expér.,' 3e sér., t. vi, 1898.
9. Brock.—"Untersuchungen über die interstitiellen Bindesubstanzen der Mollusken," 'Zeit. für wiss. Zool.,' Bd. xxxix, 1883.
10. Burne, R. H.—"A Reno-pericardial Pore in Ampullaria urceus, Mull," 'Proc. Malac. Soc. Lond.,' vol. iii, part 1, 1898.
11. Clarke.—'British Marine Testaceous Mollusca,' pp. 308, 309, 313.
12. Cunningham.—"The Renal Organs (Nephridia) of Patella," 'Quart. Journ. Micr. Sci.,' vol. 23, 1883.
13. Erlanger, R. von.—"Zur Entwicklung von Paludina vivipara," 'Morph. Jahrb.,' Bd. xvii, 1891.
14. Erlanger, R. von.—"On the Paired Nephridia of Prosobranchs," 'Quart. Journ. Micr. Sci.,' vol. 33, 1892.
15. Fischer, H.—"Recherches sur la morphologie du foie des Gastéropodes," 'Bull. Sci. France et Belg.,' t. xxiv.
16. Fleure, H. J.—"Notes on the Relations of the Kidneys in Haliotis tuberculata, etc.," 'Quart. Journ. Micr. Sci.,' vol. 46, 1902.
17. Forbes and Hanley.—'A History of the British Mollusca,' vol. ii, pp. 489—546, London, 1853.
18. Goodrich.—"On the Reno-pericardial Canals in Patella," 'Quart. Journ. Micr. Sci.,' vol. 41, 1899.

19. Haller, Béla.—"Untersuchungen über marine Rhipidoglossen. I. Studie," 'Morph. Jahrb.,' Bd. ix, 1884.

20. Haller, Béla.—"Untersuchungen über marine Rhipidoglossen. II. Studie. Textur des Central-nervensystems und seiner Hüllen," 'Morph. Jahrb.,' Bd. xi, 1866, pp. 321—436.

21. Haller, Béla.—"Beiträge zur Kenntniss der Niere des Prosobranchier,' 'Morph. Jahrb.,' Bd. x, 1886.

22. Hilger.—"Beiträge zur Kenntniss des Gastropodenauges," 'Morph. Jahrb.,' Bd. x, 1885.

23. Houssay, F.—"Recherches sur l'opercule et les glandes du pied des Gastéropodes," 'Arch. Zool. expér,' 2e sér., t. ii, 1884.

24. Jeffries, Gwyn.—'British Conchology,' vol. iii, pp. 286—336, London, 1865.

25. Jhering, H. von.—"Zu. Morph. der Niere des Sog. Moll." 'Zeit. für wiss. Zool.,' Bd. xxix, p. 583—614, 1877.

26. Lacaze Duthiers.—"Mémoir sur le système nerveux de l' Haliotidæ," 'Ann. Sci. Nat. Zool.,' 4e sér., t. xii, 1859.

27. Lacaze Duthiers.—"Otocystes ou capsules auditives des Mollusques (Gastéropodes)," 'Archiv. de Zool. expér.,' 1e sér., t. i, 1872.

28. Lacaze Duthiers.—"De l'epipodium chez quelques Gastéropodes," 'Compt. rend. Acad. Sci.,' t. c., 1885.

29. Lacaze Duthiers.—"De la valeur relative de quelques procédés d'investigation en anatomie comparée," 'Archiv. de Zool. expér.,' 2e sér, t. viii, 1890.

30. Moore and Randles.—"A New Interpretation of the Gastric Organs of Spirula, Nautilus, and the Gastropods," 'Proc. Roy. Soc. Lond.,' vol. lxx, 1902, pp. 231—237.

31. Pelseneer, P.—"Sur la valeur morphologique de l'epidodium des Gastéropodes Rhipidoglosses," 'Compt. rend. Acad. Sci.,' t. cv, No. 14, 1887.

32. Pelseneer, P.—"Sur l'epipodium des Mollusques," 1re note, 'Bull. Scientif. France et Belgique,' t. xix, 1888.

33. Pelseneer, P.—"Sur l'epipodium des Mollusques," 2me note, 'Bull. Scientif. France et Belgique,' t. xxii, 1890.

34. Pelseneer, P.—"Sur l'epipodium des Mollusques," 3me note, 'Bull Scientif. France et Belgique,' t. xxiii, 1891.

35. Pelseneer, P.—"Les reins, les glandes génitales et leur conduits dans les Mollusques," 'Zool. Anz.,' Bd. xix, No. 499, 1896.

36. Pelseneer, P.—"Recherches Morphologiques et Phylogénetiques sur les Mollusques Archaiques (Mémoirs courronés et Mémoirs des Savants étranges)," 'Acad. Roy. des Sci. de Belgique.'

37. Perrier, Remy.—"Recherches sur l'anatomie et l'histologie du rein des Gastéropodes Prosobranches," 'Ann. Sci. Nat. Zool.,' 7e sér., t. viii, 1889.

38. Robert, A.—"Le Troque (Trochus turbinatus, Born.)," 'Zoologie Descriptive des Invertebres,' t. ii, pp. 381—445, Paris, 1900 (Octave Doin, Éditeur).

39. Spengel.—"Die Geruchsorgane und das Nervensystem der Mollusken," 'Zeit. für wiss. Zool.,' Bd. xxxv, 1881.

40. Thiele.—"Beiträge zur Kenntniss der Mollusken. i, Ueber das Epipodium," 'Zeit. für wiss. Zool.,' Bd. liii, 1892.

41. Thiele.—"Beiträge zur Kenntniss der Mollusken. iii, Ueber Hautdrüsen und ihre Derivate," 'Zeit. für wiss. Zool.,' Bd. lxii, 1897.

42. Troschel, H.—'Das Gebiss der Schnecken,' Berlin, 1856—1863.

43. Tryon.—'Manual of Conchology,' vol. xi, Trochidæ.

44. Wegmann.—"Contribution à l'histoire naturelle des Haliotides," 'Archiv. de Zool. expér.,' 2e sér., t. ii, 1884.

45. Woodward, M. F.—"The Anatomy of Pleurotomaria Beyrichii," 'Quart. Journ. Micr. Sci.,' vol. 44, 1901.

EXPLANATION OF PLATES 4—6,

Illustrating Mr. W. B. Randles' paper on "Some Observations on the Anatomy and Affinities of the Trochidæ."

Reference Letters.

a. ao. Anterior aorta. *a. br.* Afferent branchial vessel. *a. oc. p.* Appendix of ocular peduncle. *a. r. k.* Anterior lobe of right kidney. *ab. g.* Abdomina ganglion. *amp.* Ampulla (enlarged portion of ureter in T. zizyphinus). *b. d* Bile-duct. *b. g.* Buccal ganglion. *b. m.* Basement membrane. *bn. g.* Branchial ganglion. *c. l.* Cephalic lappets. *c. t.* Cephalic tentacle. *cæ. g.* Cæca groove. *cb. c.* Cerebral commissure. *cb. g.* Cerebral ganglion. *cb. p.* Cerebro-pedal connective. *cb. pl.* Cerebro-pleural connective. *cl. m.* Columella muscle. *cr.* Crop. *d.* Dialyneury (left). *e. b. v.* Efferent branchial vesse

e. r. v. Efferent renal vein of right kidney. *ep.* Epipodium. *ep. c.* Cervical lobe of epipodium. *ep. n.* Epipodial nerve. *ep. p.* Epipodial papilla. *ep. t.* Epipodial tentacles. *f.* Foot. *f. c.* Formative (chitogenous) cells of tesseræ. *g.* Gill. *g. a.* Genital aperture. *g. d.* Genital duct. *g. g.* Genital gland. *int.* Intestine. *j.* Jaw. *k. c.* Kidney chamber (right). *l.* Liver. *l. au.* Left auricle. *l. c.* Labial commissure. *l. k.* Left kidney (papillary sac). *l. k. a.* Left renal aperture. *m.* Mouth. *m. f.* Muscle-fibres. *m. g.* Mucous (hypobranchial) gland. *m. ins.* Muscle insertion. *ma.* Mantle. *ma. c.* Mantle-cavity. *o. m.* Outer limiting membrane of jaw. *o. n.* Optic nerve. *oc. p.* Ocular peduncle. *od.* Odontophore. *œ.* Œsophagus. *op. l.* Opercular lobe. *os.* Osphradium. *ot.* Otocyst. *ot. n.* Octocyst nerve. *ovd.* Oviduct. *p. ao.* Posterior aorta. *p. gl.* Pedal gland. *p. n.* Pedal nerve. *p. r. k.* Posterior lobe of right kidney. *pa. n.* Pallial nerve (right). *pa. n.'* Pallial nerve (left). *pc.* Pericardium. *pd. c.* Pedal cords. *pl. g* Pleural ganglion. *pl. p.* Pleuro-pedal connective. *r.* Rectum. *r. au.* Right auricle. *r. k.* Right kidney. *r. k. a.* Right kidney aperture. *r. p. c.* Reno-pericardial canal (right). *r.' p. c.* Reno-pericardial canal (left). *r. s.* Radula-sac. *sb. int.* Subintestinal nerve, *sl. g.* Salivary gland. *sp. c.* Spiral cæcum. *sp. int.* Supra-intestinal nerve. *st.* Stomach. *t. n.* Tentacular nerve. *t. p. v.* Transverse pallial vein. *ts.* Tesseræ of jaw. *u.* Ureter. *um.* Umbilicus. *v.* Ventricle. *v. n.* Visceral nerve.

PLATE 4.

Fig. 1.—Shell of Trochus magus.

Fig. 2.—Shell of T. umbilicatus.

Fig. 3.—Shell of T. lineatus.

Fig. 4.—Shell of T. zizyphinus.

Fig. 5.—Head of T. umbilicatus, viewed from the right side. × 5.

Fig. 6.—Head and foot of T. granulatus, viewed from the left side. The anterior part of the foot is represented in section to exhibit the pedal gland. × 2½.

Fig. 7.—Trochus lineatus, viewed from the ventral surface. × 2½.

Fig. 8.—Foot of T. granulatus, seen from the dorsal surface. × 3.

Fig. 9.—Dorsal surface of the foot of T. magus. × 2.

Fig. 10.—Operculum of T. magus. × 3½.

Fig. 11.—Operculum of T. zizyphinus. × 4.

Fig. 12.—Jaws of T. zizyphinus. × 12.

Fig. 13.—Jaws of T. magus. × 25.

Fig. 14.—Transverse section of the jaw of T. zizyphinus. × 250.

FIG. 15.—Radula of T. magus; portion of a single transverse row of teeth. × 75.

FIGS. 16 AND 17.—Radula of T. magus; marginal teeth. × 75.

FIG. 18.—Radula of T. tumidus; portion of a transverse row of teeth. × 250.

PLATE 5.

FIG. 19.—Radula of Trochus lineatus; portion of a transverse row of teeth. × 75.

FIG. 20.—Radula of T. zizyphinus; part of a transverse row of teeth. × 75.

FIG. 21.—Radula of T. granulatus; part of a transverse row of teeth. × 75.

FIGS. 22—24.—Marginal teeth of T. zizyphinus. × 75.

FIG. 25.—Marginal tooth of T. granulatus. × 75.

FIG. 26.—Marginal tooth of T. zizyphinus. × 75.

FIG. 27.—Flabelliform teeth of T. zizyphinus. × 75.

FIG. 28.—Radula of T. striatus; part of a transverse row of teeth. × 250.

FIG. 29.—Radula of T. exasperatus; part of a transverse row of teeth. × 250.

FIG. 30.—Diagram of the nervous system of T. cinerarius, viewed from above.

FIG. 31.—Transverse section through the anterior portion of the ventral (pedal) nerve-cord of T. cinerarius (right side). × 75.

FIG. 32.—Transverse section through the middle region of the pedal nerve-cords of T. umbilicatus, passing through the anterior epipodial nerve. × 75.

FIG. 33.—Transverse section through the anterior region of the pedal nerve-cords of Trochus. × 75.

FIG. 34.—Longitudinal section through the papillary sac and left reno-pericardial canal of T. magus (semi-diagrammatic). × 12.

FIG. 35.—Section (oblique) through the pericardium and kidneys of T. magus, showing the right reno-pericardial pore and the genital duct. × 15.

FIG. 36.—Section (oblique) through the pericardium and kidneys of T. magus, showing the genital duct (oviduct) opening on a small papilla into the ureter (or right kidney-chamber). × 15.

FIG. 37.—Section through the right kidney of T. magus. × 400.

FIG. 38.—Section through part of the left auricle of T. magus, passing through the pericardial gland. × 350.

PLATE 6.

FIG. 39.—General dissection of T. magus from above. The mantle has been cut along the middle line up to the pericardium, each half being reflected; the floor of the mantle-cavity and dorsal surface of the head have been removed to show the arrangement of the viscera. × 3½.

FIG. 40.—General dissection of T. lineatus from the right side. The mantle has been cut on the right side, close to the body-wall, and reflected to the left. The body-wall has been removed from the right side of the head and body. × 3.

FIG. 41.—Pallial complex of T. magus. The mantle has been cut along the right and left sides and removed from the body; the pericardium, heart, and part of the right kidney being removed with it. × 2.

FIG. 42.—Pallial complex of T. lineatus, removed from the body as above. × 2.

FIG. 43.—Pallial complex of T. zizyphinus. × 3.

FIG. 44.—Side view of the buccal mass of T. zizyphinus, showing the salivary gland, cerebral ganglia, buccal nerves, and labial commissures. × 3.

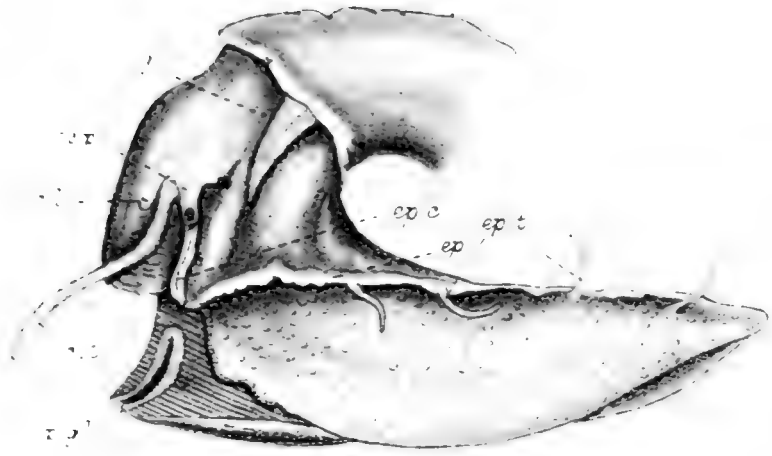
FIG. 45.—Stomach of T. lineatus opened to show internal structure. × 5.

FIG. 46.—Section through the stomach of T. lineatus. × 350.

FIG. 47.—Heart of T. magus, seen from above. The roof of the pericardium has been removed. × 4.

FIG. 48.—Pericardial cavity of T. magus; the heart and rectum have been removed together with the roof of the pericardium. The apertures of the two reno-pericardial canals are seen on the left side, and the large efferent renal vein on the floor of the pericardial cavity. × 5.

FIG. 49.—Dissection of the right kidney of T. zizyphinus, showing the anterior and posterior lobes, the ampullary enlargement of the ureter, also the opening of the oviduct into the ureter (semi-diagrammatic). × 3.



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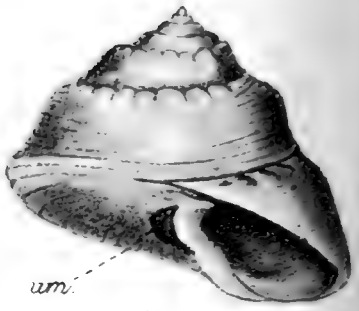

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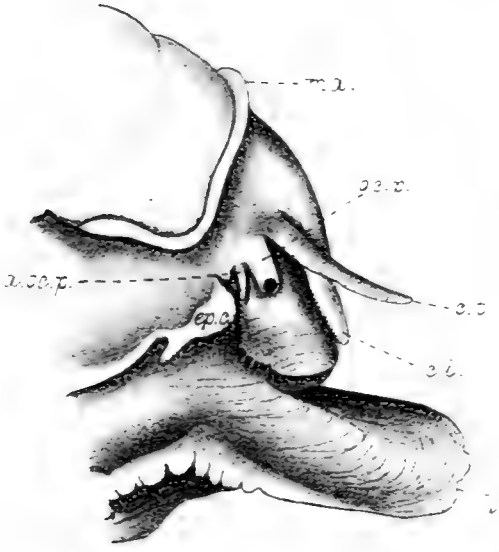

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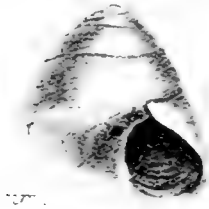

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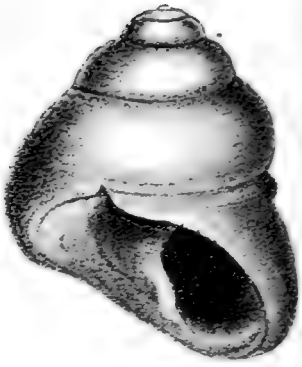

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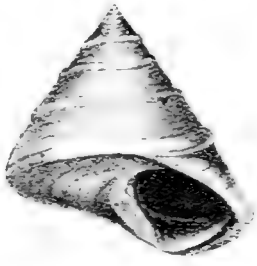

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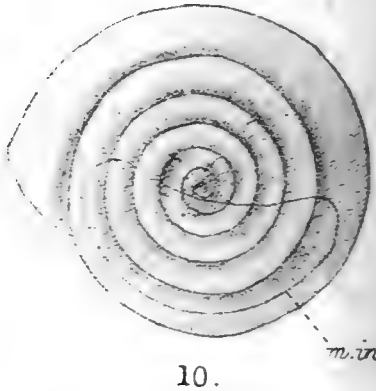

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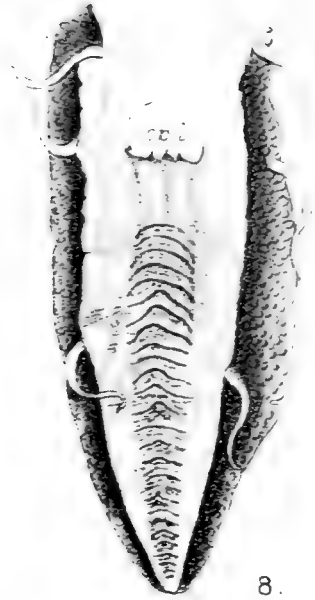

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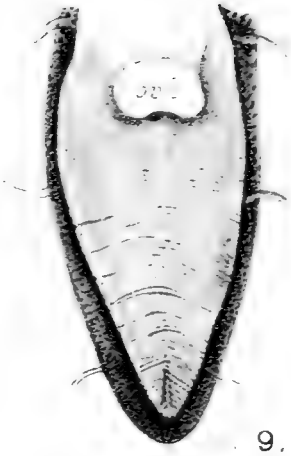

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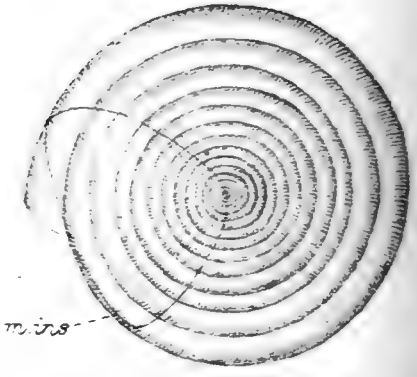

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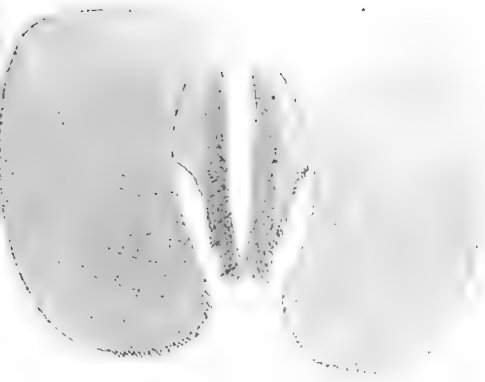

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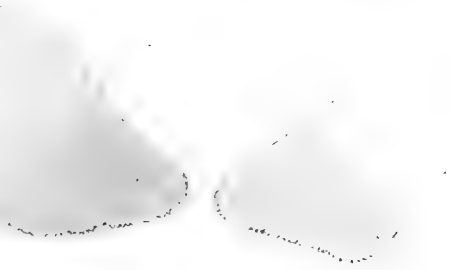

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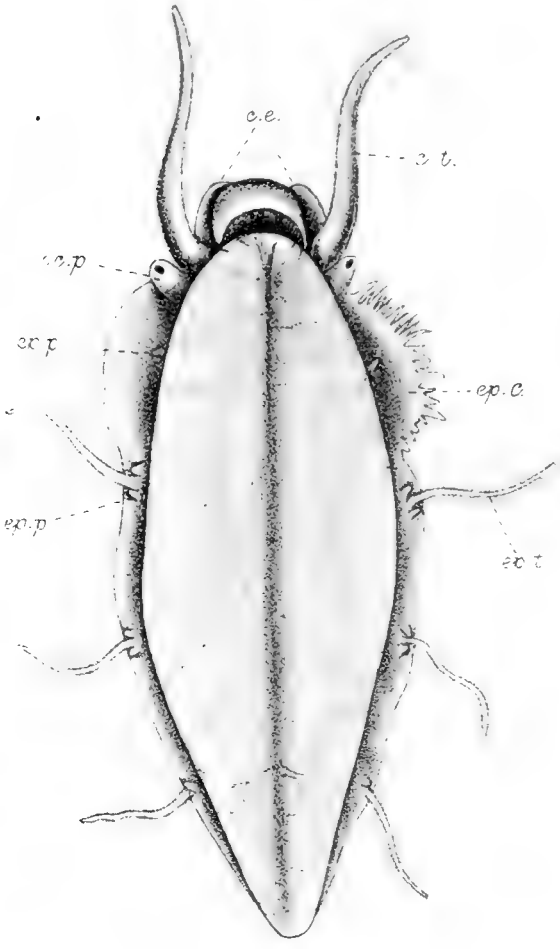

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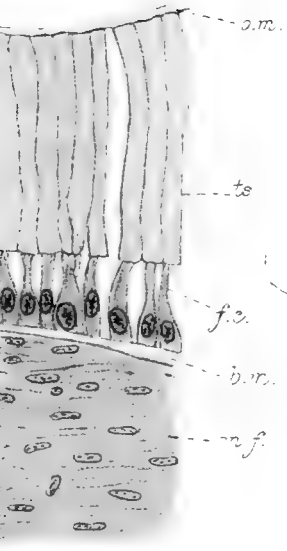

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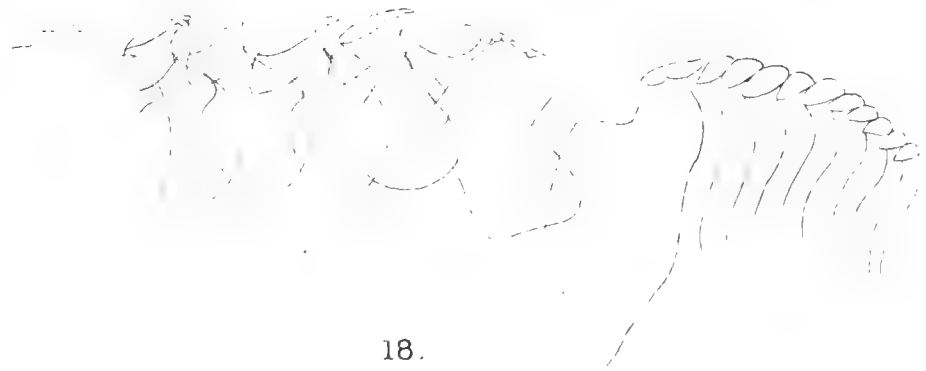

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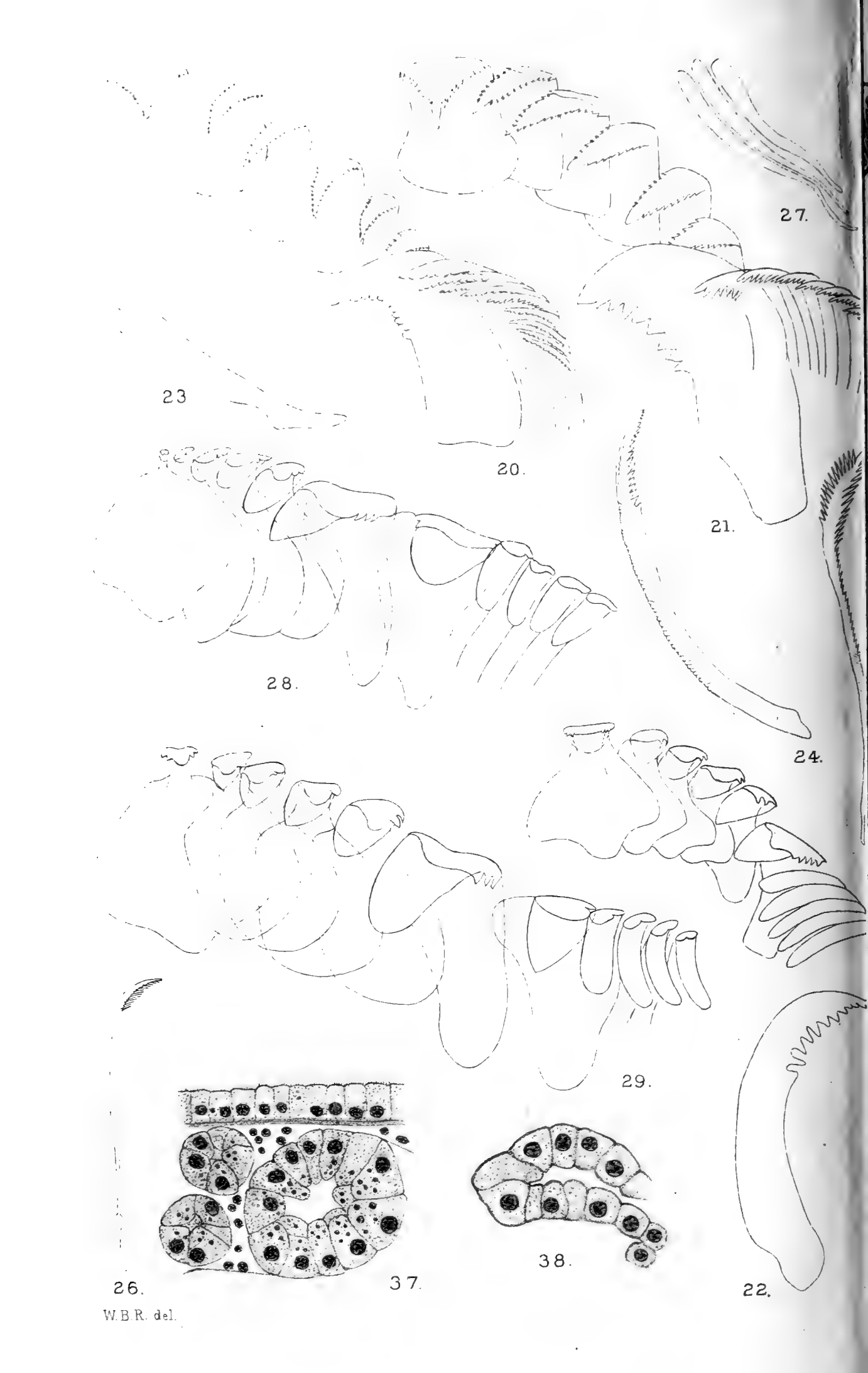

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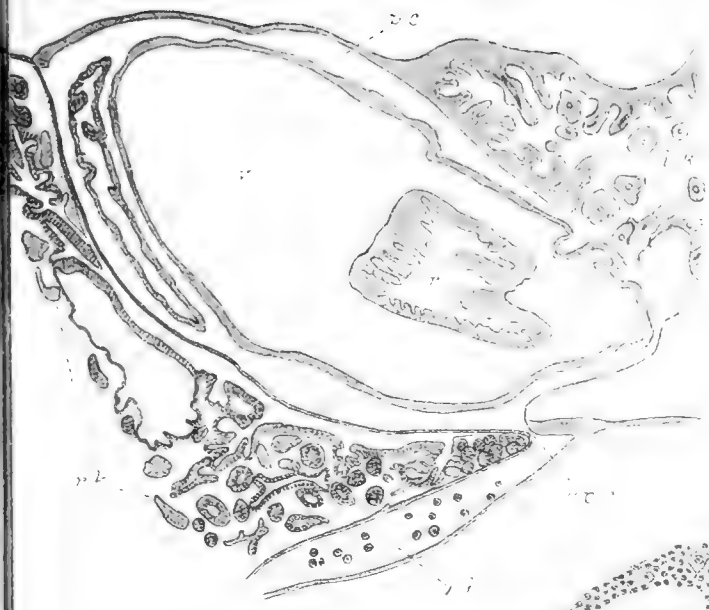

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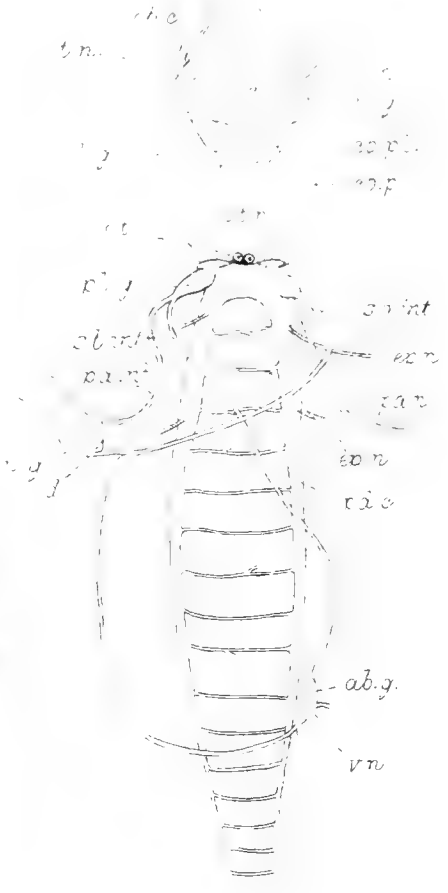

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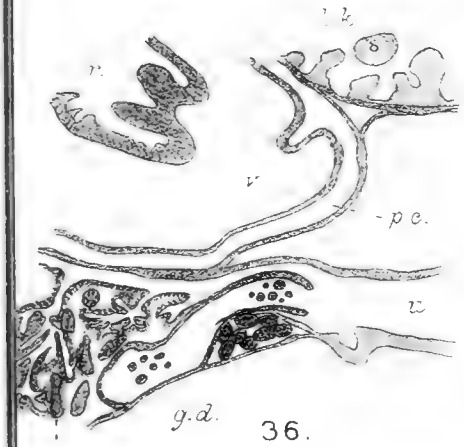

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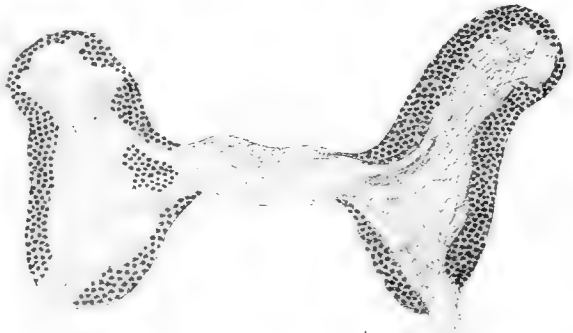

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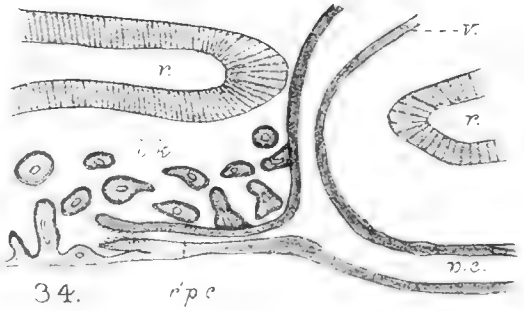

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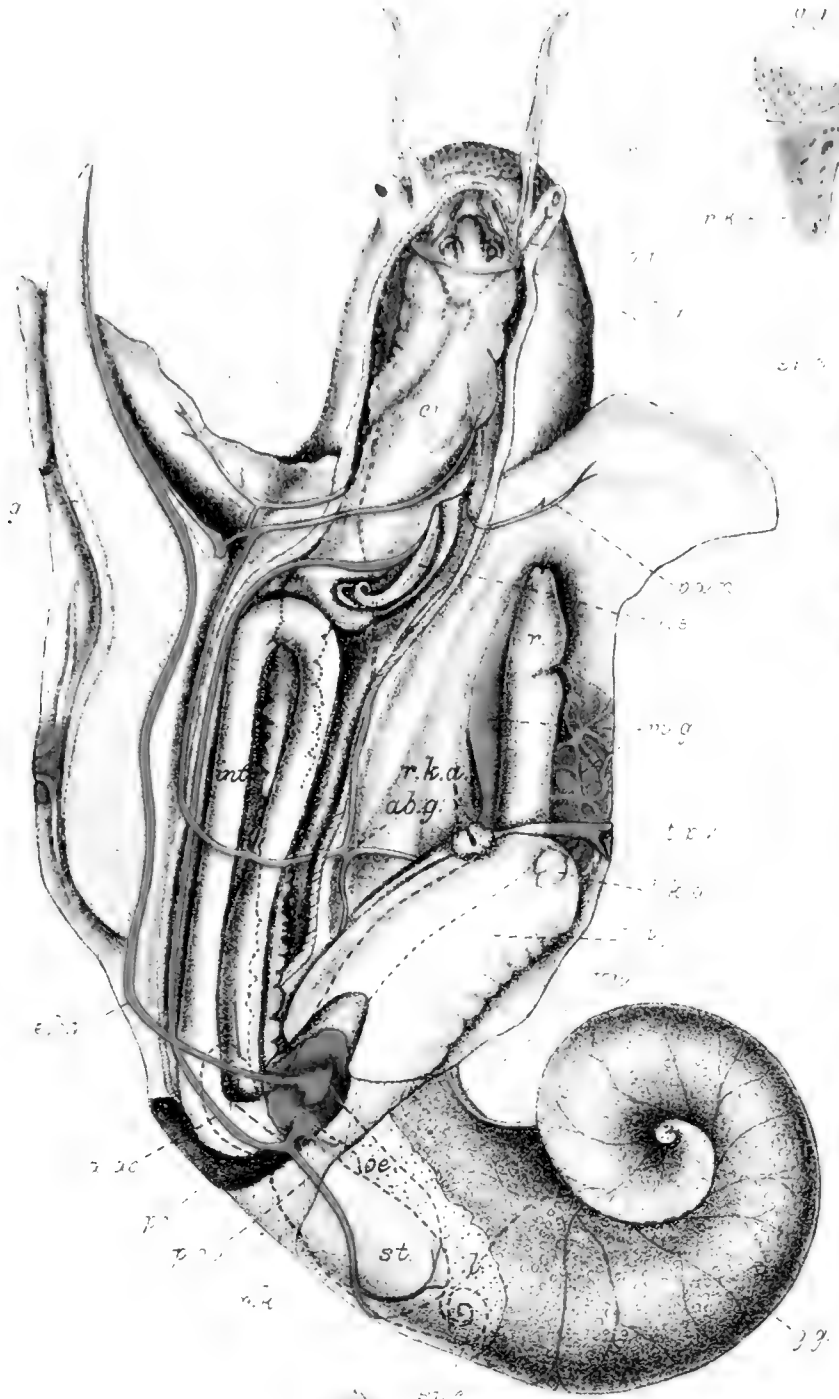
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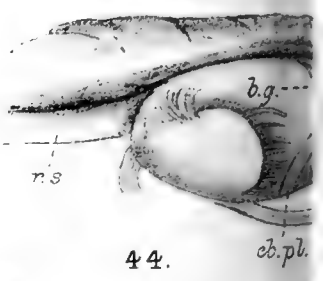
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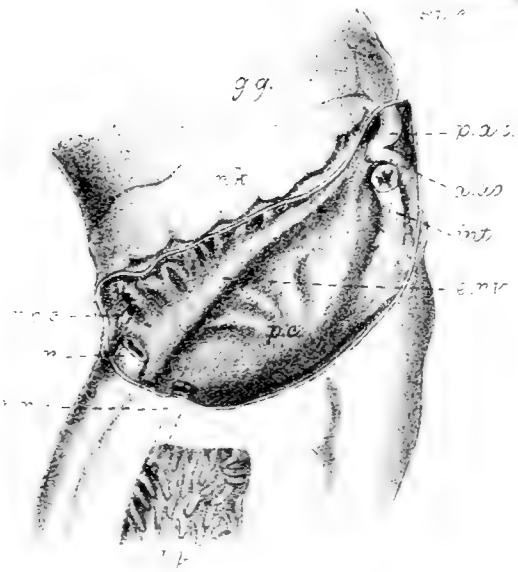
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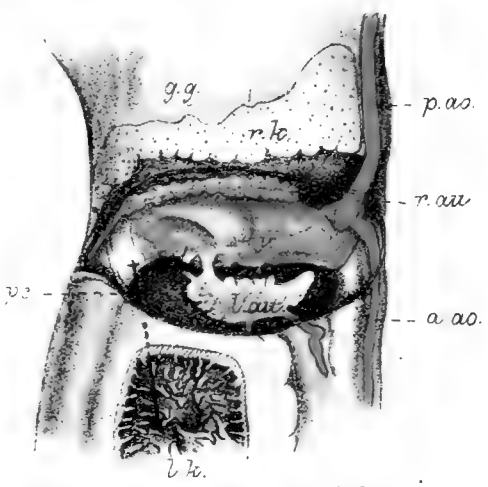
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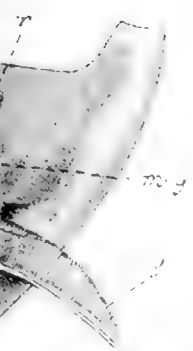
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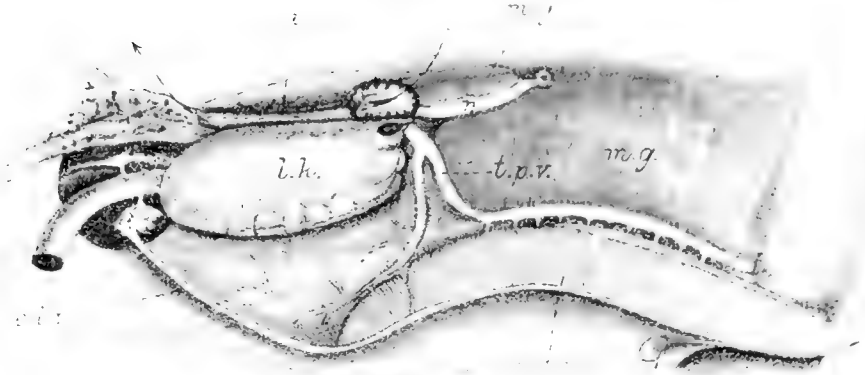
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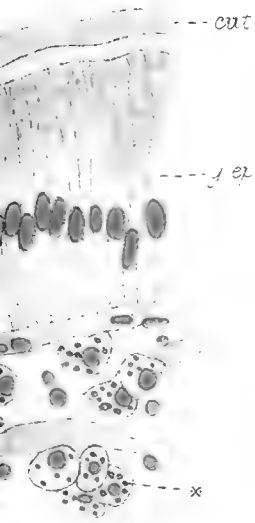
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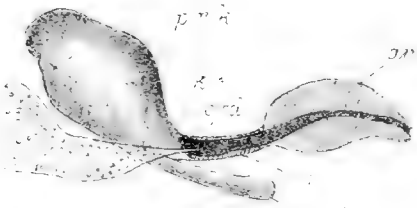
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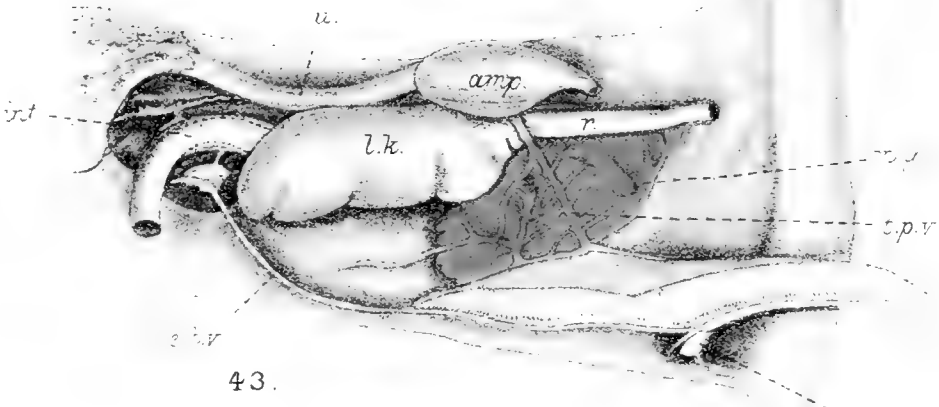
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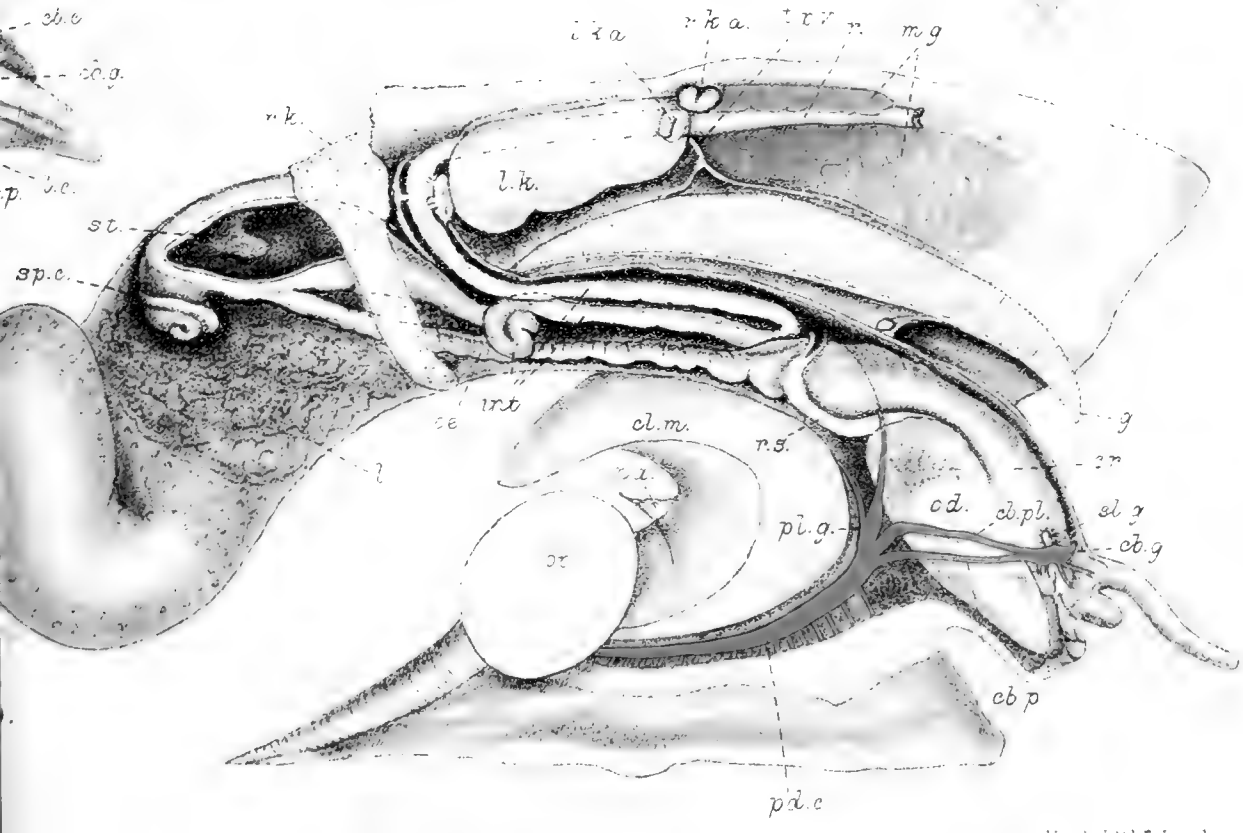
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The Anatomy of Pœcilochætus, Claparède.

By

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With Plates 7—12 and one Figure in the Text.

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Historical.

Claparède, in his 'Beobachtungen über Anatomie und Entwicklungsgeschichte wirbelloser Thiere an der Küste von Normandie Angestellt,' published in 1863, describes and figures (pp. 77—80, Taf. vi, figs. 1—11) several stages in the development of an annelid larva, which he was unable at the time to assign to any known genus. This larva was very common in the plankton at St. Vaast, and the same, or a very similar one, had previously been found (in 1855) by Claparède on the coast of Norway. He surmised that the larva must belong to some common worm at that time still undescribed.

No further advance seems to have been made in the knowledge of this form until the appearance in 1874 of a report by Claparède on the annelids collected by the "Lightning" Expedition. This report is contained in Ehler's paper, "Beiträge zur Kenntniss der Verticalverbreitung der Borstenwürmer in Meere" (Ehlers, 1874). Amongst the material collected by the "Lightning," Claparède found a number of fragments of a worm, which he considered must be the adult form of the larva he had previously described. He states that the species is represented in the "Lightning" material "par un fragment dans les préparations Nr. 15 et Nr. 24, et par tous les fragments inclus dans la préparation Nr. 22." The localities from which these specimens were obtained are not mentioned. In the same paper Ehlers refers to two fragments of the worm described by Claparède, which he found amongst the material dredged by the "Porcupine." According to the table given (loc. cit., p. 25), these were dredged on July 21st, 1869, at 48° 51′ N., 11° 7′ W. (11° 9′ W.) in 725 fathoms, on a bottom of muddy sand.

From the fragments at his disposal Claparède was able to give a fair account of the general external features of the worm, and to convince himself that it was the adult form of the larva which he had previously described, or at any rate closely allied to the adult of that larva. He gives to the worm the name Pœcilochætus fulgoris, both the generic

and the specific name being new. He was still unable to include it in any known family, and thought it not improbable that a special family would have to be made to receive it. Figures are given (loc. cit., Taf. i, fig. 1, A, B, C, and D) of the head end from the dorsal and ventral surfaces, of several chætæ, of a parapodium, and of the external opening of one of the epithelial glands, the latter being described as "petits tubercules granuleux."

Levinsen (1883, p. 106) gives some further details of the structure of late larval stages of Pœcilochætus from observations upon specimens which had been taken by the "Hauch" Expedition in the Skager Rack. He also discusses the relations of Pœcilochætus with Disoma multisetosum, Oersted, and points out that the two genera are closely allied. He places both genera in the family Spionidæ.

McIntosh (1894) furnishes some notes, accompanied by four figures, on the larva described by Claparède. He considers that the first notice of this larva is due to Maximillian Müller (1852), but reference to Müller's paper has not convinced me that the tail end of a larva which he figures is really the same as Claparède's larva.

McIntosh makes no mention of Claparède's discovery of the adult Pœcilochætus, nor of Levinsen's discussion of the subject. He states that the larva occurs in considerable numbers in the bottom-nets at St. Andrews from July to October. McIntosh gives a figure of an advanced larval stage, showing the two palps well developed.

Mesnil (1897), in his monograph on the Spionidæ, discusses the position of Pœcilochætus in relation to that family. He proposes to place it with Disoma in a new family, the Disomidæ (see further, p. 140).

Occurrence at Plymouth.

The larva of Pœcilochætus has been constantly and regularly taken for many years in the plankton collected at Plymouth during the summer months, though I believe no

record of the fact has ever been published. The larva is probably frequent in plankton taken all round our coasts, and its appearance will be well known to workers, as it renders itself conspicuous by its rapid, wriggling motion and by the row of pigment spots (large branching chromatophores) between the parapodial cirri along each side of the body.

On April 10th, 1902, the Laboratory fisherman brought in two specimens of a worm which he recognised as unfamiliar. These specimens he had obtained when digging on a patch of sand exposed at low spring tide immediately south of the coastguard station at Mount Batten, on the eastern side of Plymouth Sound. The worm has proved to be the adult Pœcilochætus, which forms the subject of the present paper.

Since that time I have always been able to obtain a few specimens whenever the tide has allowed of digging on this particular patch of sand. Unfortunately the sand is only uncovered at the lowest spring tides, and it is only on comparatively few days during the year that the worm can be obtained. During the hour, or hour and a half, that the sand may be uncovered at any tide from six to eight head ends of the worm have been collected. As the animals break very readily when disturbed, complete specimens are difficult to procure, and only two such have as yet been obtained. The local area of distribution of Pœcilochætus is very restricted. The portion of shore where it is known to live consists of patches of sand covered with zostera, with intermediate patches of a somewhat different texture on which no zostera grows. The worm appears to live only in these intermediate patches, and never in the zostera beds. It has never yet been obtained from any other locality in the Plymouth district.

I propose for the species of Pœcilochætus found at Plymouth and described in this paper, the name Pœcilochætus serpens, the specific name being selected to indicate the rapid, wriggling movement both of the larva and of the adult worm when swimming.

Habits.

Pœcilochætus serpens constructs U-shaped tubes in fine sand. These tubes are lined with a stiff layer of fine particles of mud or clay held together with mucus. The worm in its tube is shown in fig. 12 (Pl. 9). This drawing, of natural size, was made from a tube which had been constructed by a worm in a glass cell formed of two glass plates lying about $\frac{1}{16}$ inch apart and partially filled with sand. The process of burrowing was carefully watched, and the animal remained under observation in its tube for some hours. The burrowing was accomplished with the head end of the worm, more particularly with the forwardly directed parapodial cirri of the first segment and the long bristles belonging to it. During the process the anterior part of the body was constantly waved to and fro in a transverse direction. The burrowing movement was persisted in until the complete U-shaped tube had been formed.

When at rest the animal lies in its tube either with the two long palps extended in front, the ends being often protruded for some distance beyond the opening of the tube, or with the palps lying in a number of loose coils immediately in front of the head. A constant current of water, drawing small particles with it, is kept up through the tube by means of an undulatory movement of the body and of a fan-like movement of the parapodia and bristles. The movement of the numerous feather-like bristles in the posterior part of the body (Pl. 9, fig. 10) plays an important part in the production of the current that enters the tube at the end towards which the head of the worm is directed, and passes backwards over the body. If the animal reverses its position in the tube, which frequently happened in the specimen under observation, the direction of the current is immediately reversed.

As the worm possesses no jaws, it seems probable that its food consists entirely of fine organic particles and of small organisms carried in the current which it sets up. This is

confirmed by the appearance presented by food-masses in the intestine, as seen in sections of preserved material, which generally show skeletons of diatoms, etc.

When removed from its tube and irritated, Pœcilochætus often swims with a rapid, serpentine motion, which recalls the motion of the larva.

Specimens were easily kept alive for some weeks in the Laboratory when provided with sand in which to construct their tubes, and worms which through injury had lost the posterior part of their bodies generally regenerated new tail ends of characteristic structure.

Pœcilochætus appears to breed practically the whole year round. Specimens were taken in February, April, May, June, August and December, and on all occasions some were found to contain almost or quite mature eggs or spermatozoa. The mode in which the eggs are laid has not been determined. The larva of Pœcilochætus is remarkable for the late stage of development to which it retains the pelagic habit.

Methods.

As careful a study as possible was made of the living worm. For further examination specimens were preserved by the methods to be described. The worms were anæsthetised by the gradual addition of alcohol to the sea-water in which they were living. They were then placed on a glass plate and killed by dropping on to them a small quantity of the preserving fluid to be employed, the worms being kept straight and extended with camel's-hair brushes until contraction had ceased. They were then transferred to a large quantity of the fixing fluid and allowed to harden.

The most successful fixation was obtained with Hermann's fluid, in which the specimens were allowed to remain from five to twelve or fourteen hours. The shorter time gave rather better results for the epithelial structures, especially the nuchal organ and lateral sense-organs, whilst the longer time was rather better for internal parts.

Good results were also obtained by the use of corrosive sublimate-acetic mixture (3 : 1) for three or four hours, the specimens being then rapidly rinsed in water and at once transferred to 70 per cent. alcohol, to which tincture of iodine was added.

Staining was for the most part done with Gustav Mann's methyl-blue-eosin mixture (Mann, 1902), sections being allowed to remain in the mixture overnight, rinsed with water, and differentiated in absolute alcohol. This method gave very excellent results with both Hermann and corrosive sublimate preservation. The formula for the stain is—

1 per cent. Methyl blue . . .	35 c.c.
1 „ „ Eosin	45 c.c.
Water	100 c.c.

Heidenhain's iron-hæmatoxylin was also employed, but, excepting for some few special points, I do not consider the resulting preparations nearly so good as those obtained by the simpler methyl-blue-eosin method.

Embedding was done in paraffin. Transverse, horizontal and sagittal sections, 4 μ and 5 μ in thickness, were cut with the Jung microtome, and fixed to the slide with distilled water to which a trace of albumen had been added.

I take this opportunity of acknowledging my very great indebtedness to Mrs. Sexton for the drawings which she has made, with remarkable skill and accuracy, of the external features of the animal, as well as of some of the sections.

External Characters.

The **body** of Pœcilochætus serpens is long and slender, narrowing posteriorly. A specimen about 55 mm. long, when alive and extended, was from 1·5 to 1·7 mm. broad (not including the parapodial cirri) in the anterior region, and consisted altogether of about 110 segments. The body is divided into a number of regions, which will be described in detail subsequently (see p. 138).

The **colour** of the anterior segments (1—15) varies from

bright scarlet to deep purple-red according to the degree of aëration of the blood, which, showing through the transparent body-walls, gives its own colour to this region (see p. 126). The parapodia and their cirri are here almost colourless. The posterior part of the body is black or dark green and white, the dark colour being due to pigment in the cells of the intestine; the white, which is specially marked in ripe males, to the genital products.

The **head** is small and hemispherical, as can be seen from the dorsal view (Pl. 7, fig. 1, and Pl. 8, fig. 7) and from the ventral view (Pl. 8, fig. 8). It is provided with four eyes, two small dorsal and two larger ventral. A short **median tentacle** has its origin on the ventral side of the head, being placed so far back that when the proboscis is completely withdrawn into the body, the base of the tentacle also comes to lie actually within the mouth (Pl. 8, fig. 8). The tentacle, which is covered with minute papillæ (the external openings of epithelial glands), extends for a short distance beyond the anterior margin of the head (figs. 1 and 7). As will be shown later, the single median tentacle represents two lateral tentacles fused together, for it receives two nerves, one from either side of the brain.

The very large **palps** (*plp.*) arise between the head proper and the parapodia of the first segment. These palps are capable of great extension (cf. Pl. 9, fig. 12), and may attain a length equal to at least half the length of the body. Their general appearance can be seen from figs. 1 and 7. They are horse-shoe shaped in transverse section, are richly supplied with papillæ, and a crenated membrane runs along each margin of the flattened side. A single large blood-vessel, along which in the living worm a constant succession of strong pulsations is seen to pass, extends through nearly the whole length of each palp.

In describing the habits of the worm it was stated (p. 83) that when the worm is in its tube the palps may either lie straight in front of the head, being often protruded out of the mouth of the tube, or they may be formed into a number of

oose coils lying immediately in front of the head. They clearly serve, amongst other functions, as important organs of respiration.

From the posterior dorsal region of the head three long tentacle-like processes arise, a long median process, which falls back on the dorsal surface of the body, and two lateral processes, the three being united into one broad base, which is attached to the head. These three processes constitute the **nuchal organ** (fig. 1 and fig. 7, *nuch.*), the very great development of which is one of the most striking features of the genus Pœcilochætus. Occasionally a specimen is seen in which one or other of the three processes has further divided, or rather given off a well-developed lateral branch. The nuchal organ is generally of a brownish colour in the living worm.

The **first segment**, or prostomium, is greatly developed, and its parapodia and chætæ are directed forwards. Each parapodium consists of a neuropodium and a notopodium completely united together, and carries a neuropodial and a notopodial cirrus, the former being large, flask-shaped and directed forwards, whilst the latter in this first segment is small and rudimentary, showing merely as a small projection on the dorsal surface of the parapodium (Pl. 8, fig. 7).

There are two bundles of simple, long, smooth chætæ, which extend for a considerable distance in front of the head. The notopodial chætæ are about twice the length of the neuropodial, and both sets curve inwards, the longest ones often crossing their fellows of the opposite side.

The parapodia and their cirri are covered with small papillæ, at the ends of which are the external openings of mucus glands. Between the neuropodial and notopodial cirrus lies a well-developed lateral sense-organ, similar in structure to those found on all the anterior segments of the body. These organs have the appearance of small, projecting, pear-shaped lobes, with the narrowest portion at the point of attachment to the parapodium. A number of sensory

hairs can be seen projecting from a cup-like depression at the outer extremity of the lobe.

The **mouth** (fig. 8) lies on the ventral surface of the first segment. It is bordered posteriorly and laterally by large cushions or lips, which are distinctly ridged, whilst anteriorly it is limited by the base of the median tentacle, of which a portion may actually lie within the mouth, when the proboscis is completely retracted.

The **proboscis** is seldom protruded; indeed, I have only seen it thus on one occasion. It was then short and broad, almost spherical in shape, and appeared to carry the median tentacle on the base of its anterior wall.

The **second segment** is only a little less developed than the first, and the parapodia with their cirri still tend to be directed forwards. The neuropodial cirrus is similar in shape to that of the first segment, but is slightly smaller. The notopodial cirrus, unlike that of the first segment, is well developed, being of about the same size as the neuropodial. Between the two cirri is a well-developed lateral sense-organ, like that on the first segment.

The notopodial chætæ spring from a chætal sac situated immediately at the base and in front of the notopodial cirrus, which may itself be said to form the posterior lip of the sac. The anterior lip of the chætal sac is broad and short. The majority of the notopodial chætæ are long, slender, and unjointed, having the form of simple, smooth hairs. At least one bristle, however, on each side in this second segment belongs to another type, being provided with rows of short spines, the type being the same as that found in segments 7 to 16 (cf. Pl. 3, fig. 15). The neuropodial chætæ (fig. 9) consist of three (or sometimes four, the fourth being rudimentary) [1] short, stout, slightly curved hooks, which arise immediately in front of the neuropodial cirrus. In addition to these hooks a few very fine, hair-like bristles occur, which are best demonstrated in sections.

[1] In sections the rudimentary fourth hook can always be seen, though it seldom pierces the skin.

The **third segment** resembles the second, excepting that the cirri are slightly smaller and more conical in shape, and there is not quite such a tendency for them to be directed forwards. The neuropodial chætæ consist of three well-developed and one rudimentary stout hooks and a few fine hairs, all as in segment 2 (Pl. 7, fig. 2). The notopodial chætæ are all smooth hairs, no spiny bristles like those in segment 2 being present.

In the **fourth segment** the cirri are not quite so large as in the third, and are usually directed outwards or slightly backwards. The chætæ of the neuropodium are no longer stout hooks, but form a bundle of straight, smooth bristles, similar to those of the notopodium. There are no spiny bristles.

The **fifth segment** (figs. 1, 3, and 7) differs from its neighbours in the fact that the neuropodial cirri are short, whilst the notopodial cirri are long and slender, being the longest cirri, with the exception of those on the first segment, which are found on the whole body of the worm (fig. 3). These two long cirri are also often carried in a somewhat different position from those on other parts of the body, being arched over the back of the worm.

The **sixth segment** closely resembles the fourth (fig. 1), the cirri being generally directed backwards. The chætæ from the third to the sixth segment are all smooth hairs, amongst which no spiny bristles are found.

Segments 1 to 6 may be considered as constituting the first sub-division of the anterior region of the body. With segment 7 a change takes place, which is expressed both in the external and internal structure of the worm. Externally—that is to say, regarded from the point of view of the structure of the parapodia only—the second sub-division of the body would seem to comprise the **segments from the seventh to the thirteenth**, but, as will be shown later (p. 139), this does not quite agree with the division indicated by the internal anatomy, which points rather to segments 7 to 11 only being classed together.

The peculiarity of the parapodia of segments 7 to 13 (figs. 4 and 5) lies in the form and structure of the notopodial and

neuropodial cirri. These cirri are flask shaped, but the basal part of each cirrus or body of the flask becomes swollen and almost spherical, whilst the neck is thin, elongated and nearly cylindrical, with a slight enlargement at the distal end. The whole cirrus, including the neck, is very rigid, being much less flexible than the cirri of the other segments, and only moves from its base at the point of attachment to the body of the worm. The stiff movement of the cirri gives a characteristic appearance to this region of the body in the living worm. The chætæ in these segments are of two kinds, smooth, slender hairs (Pl. 9, fig. 13), which show longitudinal striation under a high power, and spiny bristles (Pl. 7, figs. 4 and 5; Pl. 9, fig. 15), the number of the latter being few in each bundle.

Lateral sense-organs in the form of pear-shaped papillæ are still found between the cirri, but the bases of the papillæ, where they are attached to the parapodium, are broader than in the more anterior segments.

In **segments 14, 15 and 16** (Pl. 9, fig. 9) the parapodia have a structure more nearly resembling that found in the fourth and sixth segments. The cirri are shorter and stouter, nearly conical in shape, and are without the long stiff necks found in the segments immediately in front. The chætæ remain of two kinds, as in the latter segments, and the lateral sense-organ still protrudes from the surface of the parapodium.

With **segment 17** there is again a change, but the structure then found continues in its essential features, with the exception of the addition of gill filaments commencing at segment 21, until about thirty segments from the end of the body.

Both the notopodial and neuropodial cirri, conical in shape, are now much smaller in size (figs. 1, 10, and 11), and vary considerably and somewhat irregularly in the extent to which they are developed from segment to segment.

There is, on the other hand, a very remarkable development of the chætæ. In both notopodium and neuropodium the

smooth, slender chætæ of the anterior segments are replaced by large, hairy, feather-like bristles (Pl. 7, fig. 3; Pl. 9, figs. 10, 14, and 16), the most dorsal and most ventral in each segment having long, fairly stiff shafts, with lateral hairs of moderate length (fig. 14), whilst the inner ones (ventral bundle of notopodium and dorsal bundle of neuropodium) are more slender and flexible, but have very much longer hairs (fig. 16). These bristles give to the region of the body now under consideration a kind of woolly appearance.

The spiny bristles of the anterior segments also undergo a special modification in this region. The stoutness of their shafts becomes very greatly reduced, the spines themselves become much elongated, show a slight thickening near the tip, and are connected with the shaft along almost their entire length by a thin, transparent membrane, which is practically invisible in fresh material, but becomes quite obvious after staining (Pl. 9, fig. 17). By this arrangement the surface of the bristle becomes very greatly extended.

The hairy, feather-like bristles, together with the modified spiny bristles just described spread out in each parapodium into a large fan, the movements of which are mainly responsible for the current of water which the worm constantly draws through its U-shaped tube (see p. 83).

In this region the lateral sense-organ no longer has the form of a papilla protruding from the face of the parapodium, but is seen as a slight depression from the centre of which a bundle of sensory hairs arises. The depression is surrounded by a circular rim, which rises slightly above the general face of the parapodial surface.

Gills.—The gill filaments commence on segment 21, and are found on the succeeding segments to quite near the end of the body. They are at first short and small in size (Pl. 7, fig. 1), but soon become longer and larger. When fully developed they consist of long filaments, as long as or longer than the cirri of the parapodia (Pl. 9, fig. 11), which appear bright red in the living worm from the colour of the blood which is in them. Two pairs of such filaments occur upon

each parapodium, one pair being attached to the posterior face of the neuropodium and one pair to the posterior face of the notopodium.

The terminal segments (Pl. 8, fig. 6) show certain special features. The general shape of the body is here flattened, and the dorsal surface is somewhat concave. The neuropodial and notopodial cirri are of about the normal shape, but the neuropodial is double the size of the notopodial, and the latter assumes a more dorsal position than usual. The more dorsal of the notopodial chætæ are transformed into strong hooks (figs. 6 and 19), which form a transverse row on either side of the dorsal surface of the segment. Five or six such hooks are generally found on each notopodium. The curve of the hook is directed backwards, and those nearest the middle line are the stoutest as well as the most strongly curved. These hooks are found on the last sixteen or seventeen segments (in full-grown specimens), and obviously serve the purpose of enabling the worm to hold itself firmly in the tube.

The remaining chætæ of the notopodium and those of the neuropodium in these segments are mostly either of the ordinary smooth or spiny kinds, the latter being often rudimentary. There is also found in the terminal region of the body a special kind of bristle not met with elsewhere (Pl. 9, fig. 18). This consists of a stout, smooth shaft, showing longitudinal striations, and ending in a blunt tooth directed slightly outwards. From the base of this tooth there arises a hairy terminal portion of the bristle, which forms a kind of flexible brush attached to the end of the stiff shaft. Bristles of this character are a modified form of the ordinary stout, hairy bristles, which, as the end of the body is approached, at first lose the hairs along the greater part of the length of the shaft, retaining them only at the ends. The type of bristle with the hairy flexible end (fig. 18) becomes established at about the thirtieth segment from the end of the body (in full-grown specimens), and occurs in the segments from this point to about the ninth or tenth from the end.

In the terminal segments the lateral sense-organs have

again the form of pear-shaped papillæ protruding from the surfaces of the parapodia between the cirri.

The **pygidium** is well developed; the anus is somewhat dorsal, and is surrounded by five large lobes (Pl. 8, fig. 6). There are two pairs of anal cirri, both situated below the anus, the more dorsal pair being long and slender, the more ventral pair short.

The anus and the terminal portion of the intestine are strongly ciliated, and all the cirri in the hindermost region of the body, as well as the dorsal and ventral surfaces of the body itself, are very richly provided with papillæ, at the extremities of which lie the external openings of epithelial glands.

No description of the general aspect of the living Pœcilochætus is complete without reference to the remarkable system of blood-vessels, which is visible through the tranparent body-wall (Fig. 1). A detailed account of this vascular system will be found in the special section on p. 126.

Internal Anatomy and Histology.

Epithelium and Cuticle.

The character of the epithelium differs in different parts of the body. The cells composing it may be either almost cubical, with spherical nuclei (Pl. 9, fig. 20), or they may be elongated in a direction either perpendicular (Pl. 9, fig. 21) or parallel to the body surface (Pl. 10, fig. 23). The elongated cells have oval nuclei, the long axes of which are parallel to the long axes of the cells.

Over the greater part of the body the epithelial cells are arranged in a single layer, but in isolated places, more especially on the ventro-lateral surfaces to be presently described, two layers can be recognised. The cuticle, which lies external to the epithelial cells, varies in thickness in different parts of the body.

Cells nearly cubical in shape are found on the dorsal

surface of the anterior segments (Pl. 9, fig. 20). In preparations stained with methyl-blue-eosin the cuticle is coloured blue, a thin outer layer being distinguishable by its very dark colour from the main body of cuticular substance, which is stained uniformly of a much lighter shade. The protoplasm of the epithelial cells is very distinctly granular in preparations preserved in Hermann's fluid, and the divisions between the individual cells are often strongly marked. Each cell contains a spherical nucleus with a well-marked nuclear membrane. Within the nucleus is one large mass of deeply staining chromatin and a few small, scattered particles of the same substance. The nucleus as a whole has an exceptionally clear and transparent appearance in preparations preserved in Hermann's fluid. The internal ends of the cells appear to be in immediate contact with the muscular layers of the body-wall. Towards the tail end of the animal the epithelium of the dorsal surface becomes more flattened, the individual cells are less clearly marked, and the nuclei are transversely oval (Pl. 10, fig. 23).

On the ventro-lateral surfaces of the body the epithelial cells are generally more elongated in a direction perpendicular to the body surface (Pl. 9, fig. 21 ; Pl. 10, fig. 22), and have oval nuclei in which the chromatin is present in the form of a number of deeply staining particles connected by a network, no one particle standing out so prominently as the large single mass of chromatin in the nuclei of the cubical cells of the dorsal surface. In certain spots the elongation of the cells is very great, and some of the cells have migrated inwards, so that an internal layer of nuclei can be recognised (fig. 21). In this way a pad or cushion of cells is produced, and this cushion forms the point of insertion of certain muscle-bands.

Epithelial Gland-cells.

Gland-cells opening externally by means of short, chitinous tubes which project beyond the general surface of the body

are abundant in places. In their simplest form these consist of individual cells lying amongst the cells of the epithelium. One such cell is illustrated in fig. 22 (Pl. 10). It is pear shaped, with granular protoplasm staining much more deeply than that of the surrounding cells, and with an oval nucleus, the long axis of which lies parallel to the body surface. The protoplasm at the mouth of the cell is inserted in a depression on the internal face of the chitin. The chitinous tube, which places the interior of the cell in communication with the external water, forms a conical projection on the body surface, and can also be seen to project internally for a short distance into the protoplasm of the neck of the cell.

Such simple gland-cells are not, however, very numerous. The more usual arrangement is for several cells to be associated together and to open externally through one tube. Glands of this type are especially numerous in the epithelium towards the tail end of the animal, where the tubes are situated upon raised chitinous papillæ, which form a characteristic feature in external views of the animal. These papillæ and tubes are figured by Claparède (in Ehlers, 1874), and their great abundance on the dorsal surface of the anterior segments in the specimens examined by him constitutes one marked difference between his Pœcilochætus fulgoris, obtained from deep water, and the specimens found at Plymouth near low-tide mark on the shore.

A section through such a gland opening on the dorsal surface near the tail end of one of the Plymouth specimens is shown in fig. 23 (Pl. 10). The epithelium here consists of flattened cells, with large, oval nuclei. The cuticle is comparatively thin, except in the neighbourhood of the opening of the gland. It is there greatly thickened and pushed outwards, forming a tubercle with a stout chitinous covering hollowed out internally, the internal hollow being filled with the protoplasm of the ends of the gland-cells. Through the centre of the tubercle runs the chitinous tube, which places the gland-cells in communication with the exterior, the tube

projecting to an equal extent externally beyond the surface of the papilla and internally into the protoplasm of the gland-cells.

On account of the flattened nature of the epithelium, the gland-cells, which are easily distinguished by their more deeply staining protoplasm, do not lie immediately beneath the tubercle, but are drawn considerably to one side. The nucleus of each gland-cell lies near its proximal end. It is much smaller than the nuclei of the ordinary epithelial cells surrounding it, spherical rather than oval in shape, contains a large quantity of chromatin in the form of a considerable number of large, deeply staining granules of about equal size, and is thus very readily distinguished from the nuclei of the epithelium. Usually three or four such nuclei can be distinguished lying close together in the neighbourhood of the base of each of the chitinous tubercles. In the figure (fig. 23) only one such nucleus is shown; but three were distinguished in the sections, two lying one over the other, in the section from which the figure was made, and one in the following section.

Scattered over the ventral surface of the cuticle, especially in the anterior segments of the body, a number of rounded tubercles or callosities are found. A section through two of these is shown in fig. 21 (Pl. 9). They are almost entirely cuticular structures (*cal.*), the epithelial cells only protruding for a very short distance into them. The internal, lightly staining layer of the cuticle found in this region of the body, though curved slightly outwards, is little if at all thickened. The tubercle is chiefly formed, therefore, by a great thickening of the outer or deeply staining layer of the cuticle. The character of this layer seems also to be slightly altered, for in methyl-blue-eosin preparations it takes on a deep reddish or purple tint rather than blue, and often exhibits a characteristic radial structure due to a number of deeply staining, radiating bars (fig. 21).

The appearance of these tubercles at once brings to mind those upon which stand the tubes of the gland-cells already

described. In the present case, however, no external openings can be demonstrated, unless the radial lines already mentioned really represent pores. Nevertheless an examination of a large number both of the gland papillæ and of the callosities produces a conviction that the latter are in reality essentially the same structures as the former, either in a more highly developed or in a regenerate state.

The cells lying immediately beneath the tubercle on the right-hand side of the section figured (fig. 21) are somewhat difficult of interpretation. It is possible that the long process (p) immediately beneath the cuticle is homologous with the internal portion of the tube of the gland-cells (figs. 22 and 23), and that the nucleus (n') is the nucleus of a gland-cell with which this tube communicates. I have not, however, found other sections which appear to confirm this view.

Although gland-cells are by no means uncommon in the general body epithelium, by far the largest development of such cells takes place in the dorsal and ventral cirri of the parapodia. Fig. 25 (Pl. 10), was drawn from one of the cirri from the regenerated tail end of a living worm, where the transparency of the tissue allowed the gland-cells to be seen. Fig. 24 (Pl. 10) represents a section through a cirrus from about the eighteenth segment of the body. From the latter figure it will be seen that a cirrus is crowded with a number of flask-shaped cells, the long necks of which open to the exterior through papillæ elevated above the surface of the cirrus. (In fig. 25 the long necks of the cells are not shown, the fact that they were not visible in the fresh tissue being probably due to their great transparency. In fig. 24 the actual continuity between any one cell and the external opening does not appear, but this is quite easily demonstrated in a series of sections.)

The gland-cells in the cirri appear under at least three forms, which are illustrated in figs. 26, 27, 28, and 29 (Pl. 10). The figures have been drawn from transverse sections of cirri preserved in Hermann's fluid and stained with

methyl-blue-eosin. Cells of each of the three types possess long necks opening at the exterior as above described.

Type A.—Fig. 26 represents a section of a type of gland-cell which occurs in cirri from all parts of the body. In those on the anterior segments, from 1 to 13, it is the only kind found. In the cirri of the segments behind the thirteenth cells of this character are not numerous and general, but sometimes occur towards the distal end of the cirrus (cf. fig. 24, the very dark cells). These cells stain very deeply, the protoplasm being crowded with granules, which take on an intense blue colour. There are also present a number of short rods and particles of different shapes, which are even more deeply stained than the granules. The nucleus stains red with the eosin. It contains one large mass of deeply staining chromatin (nucleolus) surrounded by a clear space. This space is bounded by a membrane, and attached to this membrane is a hemispherical cap of deeply staining substance half enclosing the nucleus. A section through this cap gives the crescent-shaped figure shown in fig. 26 (Pl. 10). The relation of the cap to the nucleolus reminds one of the relation of the yolk nucleus of the ovum to the germinal vesicle (cf. fig. 61). The remainder of the nucleus—that is to say, the space between the nuclear membrane and the nucleolar membrane and cap—is filled with a large number of small granules, stained red with the eosin, but not taking on by any means such an intense colour as the nucleolus and the nucleolar cap.

Type B.—Figs. 27 and 28 represent sections through the type of gland-cells which occupy the greater part of the bodies of the cirri on all the segments from the fourteenth backwards. The change from cirri packed with cells of Type A in segment 13 to those containing almost entirely cells of Type B in segment 14 is very marked.

Cells of this type have a ground-substance with a homogeneous appearance—or showing in preserved material at most a faint indication of a network—which stains pale blue. In this ground substance are a number of rods (sections of

the rods appear circular) which stain a deeper blue than the ground substance of their cell, but do not take on by any means the intense blue colour of the granules in cells of Type A. The nuclei of cells of Type B resemble those of Type A in general structure. The nucleolus is, however, somewhat smaller, and all the structures take on a much less intense stain. A noteworthy feature in the sections of these cells is that the cell-substance outside the nucleus contains patches of fine red granules exactly resembling the red granules seen in the nucleus itself. These patches occur more especially in immediate contact with the nucleus, and their whole appearance seems to suggest very strongly that the granules are being manufactured in the nucleus and passed out into the surrounding substance.

For a valuable summary of our knowledge of the part played by the nucleus in secretion, and a very extensive list of the papers dealing with this subject, reference may be made to a recent paper by Launoy (1903).

Type C.—In cells of the third type (fig. 29), which also occur in the cirri of segments from 14 backwards, the ground substance stains pale blue, shows a reticular structure in preparations preserved in Hermann's fluid, and contains a few deeply staining rods. The nucleus stains deeply and diffusely, but is shrunken and irregular in shape. Cells of this type are apparently those in which the process of the formation of the secretory product is complete and the nucleus no longer active. If this be so they are in reality a later stage in the condition of cells of Type B.

If fragments of the living worm are strongly irritated, a large mass of clear, transparent mucus is secreted, which is in all probability discharged from the gland-cells of the cirri above described.

For a summarised account of epithelial gland-cells of various kinds found in other Polychætes reference may be made to Eisig's monograph on the Capitellidæ (Eisig, 1887).

Palps.

The external appearance of the palps (Pl. 7, fig. 1) has already been described (p. 86). A section of the palp shows it to be a hollow tube having a large central cavity, through which the blood-vessel of the palp runs (Pl. 10, fig. 30, *p. bv.*). The walls of the tube are composed of two layers of cells, a thick outer layer of large epithelial cells (*ep.*) lined internally by a thin sheet of mesoderm cells. The nuclei of both the epidermic and mesodermic cells have a very characteristic appearance, since each possesses a single, large, deeply staining nucleolus. Occasionally a nucleus is seen with two such nucleoli of equal size, which may indicate division. The walls of the blood-vessel which runs along the length of the palp are thick, and contain nuclei similar to those of the mesodermic cells. From the pulsation of the vessel in the living worm these walls are known to be muscular.

The cavity of the palp communicates with the general body-cavity of the first segment of the worm by means of a tube formed by a continuation into the body-cavity of the mesoderm-cells lining the cavity of the palp (fig. 30, *plp. v.*). This tube, just after it leaves the palp, is surrounded by a strong band of annular muscle-fibres, by the contraction of which the cavity of the palp can be completely cut off from the body-cavity of the first segment. It is clear that the palps, which are capable of very great extension, are elongated by the pressure of fluid from the body-cavity into their cavity. When once the palps are filled, the contraction of the annular muscle-fibres just described will enable them to continue extended without the necessity of the body pressure being maintained. (For an account of the muscular septa which come into play when the fluid is pressed forward see p. 123.)

At the outer side of the palp, between the base of the palp and the palp-ganglion, lies a small diverticle (*plp. div.*) of the palp cavity, which appears to run forwards and then end blindly. The meaning of this structure I have not fully understood.

Chætæ.

An account has already been given, in describing the external features, of the different types of bristles which Pœcilochætus possesses (see Pl. 9, figs. 13—19). The shafts of these bristles almost all show longitudinal striation, together with transverse markings at irregular intervals. The longitudinal striations are shown in sections to be due to the fact that the bristle is built up of a large number of longitudinal tubes lying side by side. This is especially marked in the stout hooks of the neuropodium, which occur in segments 2 and 3, and in the hooks of the notopodium in the terminal segments. All chætæ with stout shafts also show the structure well.

This type of minute structure in the bristles of Chætopods has recently been described in detail by Schepotieff (1903), to whose paper reference should be made for further details.

Nervous System.

Brain.—Practically the whole of the head of the worm is occupied by the substance of the brain. This substance consists of a ventral[1] mass of nervous felt-work (punctated substance) covered externally by a mass of ganglion-cells.

The arrangement of the parts can be best explained by reference to the diagrammatic figure of a section of the brain given in the text (Fig. 1).

This figure represents a thick sagittal (longitudinal-vertical) section through the brain cut a little on one side of the middle line, and has been constructed from an examination of several series of thin sections. The large circumœsophageal commissures, which put the brain in communication with the ventral nerve-cord of the worm, can be traced from the particular mass of punctated substance which occupies the centre of the brain

[1] In the description of the brain the terms anterior, posterior, dorsal and ventral are used on the assumption that the worm has the proboscis slightly everted as in the sagittal section fig. 42 (Pl. 11). In the position of repose, what is here called the anterior surface, becomes more ventral in position.

(*m. b.*), it being probably here that the fibres of the commissures from the two sides cross each other. From this region the fibres pass first forwards and downwards and then turn outwards, after which, in sagittal sections, they form a circular patch of transversly cut fibres (*comm.*), which can be easily followed through the brain substance. Arising from the same central mass of punctated substance (*m. b.*), but at a level external to that at which the fibres of the commissures leave it, a bundle of fibres can be traced, which passes forwards and downwards into the epithelium in front of the brain, from whence it can be easily followed as a well-marked

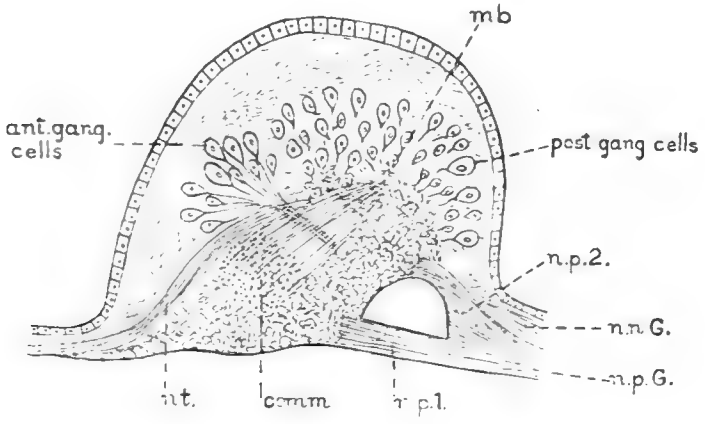


FIG. 1.—Diagrammatic sagittal section through one side of the brain of *Pœcilochætus*. *ant. gang. cells*, anterior ganglion cells; *n. t.*, nerve to median tentacle; *comm.*, œsophageal commissure; *n. p. 1*, anterior root of nerve to palp-ganglion; *n. p. 2*, posterior ditto; *n. p. G.*, nerve to palp-ganglion; *post. gang. cells*, posterior ganglion-cells; *m. b.*, central mass of fibres from which œsophageal commissures and tentacle nerve arise; *n. n. g.*, nerve to nuchal ganglion.

nerve (*n. t.*) to the median tentacle, which lies just in front of the mouth (cf. Pl. 11, fig. 42). The bundle of fibres just described exists on each side of the brain, and two nerves, one from each side, can be followed with perfect certainty from the centre of the brain to the single median tentacle.

From about the middle of the ventral surface of the brain on each side a bundle of fibres arises (*n. p.* 1) which passes backwards. This bundle of fibres is subsequently joined by a second bundle (*n. p.* 2), which leaves the brain at its

posterior end. The two bundles unite to form a stout nerve, which runs outwards to a large ganglion, situated at the base of the palp, the palp-ganglion (cf. Pl. 10, fig. 30). The nerve of the palp-ganglion thus has a double origin in the brain.

From the posterior end of the brain, fibres also pass backwards and enter the nuchal organ, where they mingle with the felt-work of fibres of a large ganglion which lies in the base of that organ—the nuchal ganglion (fig. 42). The fibres passing from the brain to the nuchal organ are not easy to demonstrate, as they do not form definite nerve-bundles, but rather two thin sheets, one from each half of the brain, the individual fibres of which pass between or below the epithelial cells lying at the junction of the nuchal organ and brain.

The ganglion-cells of the brain form an almost continuous cap covering the punctated substance. It is, however, possible to distinguish in each half of the brain an anterior group of cells (Text-fig. 1, *ant. gang. cells*), the fibres from which unite in a bundle, which is directed downwards and backwards into the mass of punctated substance lying below that region (*m. b.*), where the œsophageal commissures take origin. The further fate of this bundle of fibres could not be ascertained, but a comparison with transverse sections appears to suggest that it may cross with its fellow and then give rise to the anterior root of the palp-ganglion nerve (*n. p.* 1) of the opposite side.

A number of very large ganglion-cells (*post gang. cells*), situated at the posterior end of the brain, which send their processes into the general mass of the central felt-work, are also conspicuous. These cells, however, do not appear to constitute a definite group, but grade off into the general mass of ganglion-cells.

The Palp-ganglia.—The two palp-ganglia are situated at some distance from the brain. They are ganglia of considerable size, and contain large ganglion-cells, as well as a nervous felt-work. They lie one on each side of the body at the base of the palp and external to that structure (Pl. 10, fig. 30). Each

ganglion receives the fibres of the palp-ganglion nerve (the origin of which in the brain has already been described), and gives off a bundle of nerve-fibres, which immediately enters the palp.

The Nuchal Ganglion.—The nuchal ganglion lies in the base of the nuchal organ, and consists of a considerable mass of nervous felt-work surrounded by a number of ganglion-cells, some of them of large size. Bundles of nerve-fibres pass from it into the different branches of the organ, and these doubtless supply the external ciliated grooves which run along those branches (cf. p. 112), though individual fibres have not actually been traced so far.

The Relation of the Different Parts of the Brain to one another.—The arrangement of the brain and the ganglia connected with it in Pœcilochætus is of some theoretical interest when considered in connection with that of other Polychætes. Our recent knowledge of the structure of the Polychæte enchaphalon is largely based on the careful work of Racovitza (1896). This author distinguishes three regions, to which he gives equal morphological importance—the fore-brain (Cerveau antérieur), with which the palp-ganglia are connected; the mid-brain (Cerveau moyen) with the antennary and optic ganglia; and the hind brain (Cerveau postérieur) with the nuchal ganglia. The relations of the parts in Pœcilochætus are noteworthy in that the palp-ganglia and the nuchal ganglion are not fused in the mass of the brain, as in the forms described by Racovitza, but are separated distinctly from it. The eyes being simple, there is no development of optic ganglia, and the antennary ganglia are also not obvious. With regard to the divisions of the brain itself, the facts point to the presence of the first two at any rate of Racovitza's three regions, though the matter is by no means clear. The anterior ganglion-cells (Text-fig. 1, *ant. gang. cells*), with their bundle of fibres, which, as has been stated, very possibly form the first root of the palp-ganglion nerve, would represent the fore-brain of Racovitza, whilst the fact that the nerves to the tentacle (*n. t.*) and the œsophageal commissures

all spring from the same point in the centre of the brain (*m. b.*) would seem to point to this region as the mid-brain of that author. With regard to the hind brain, there is more difficulty. Judging from Racovitza's figures of Eurythœ borealis and Euphrosyne Audonini, it would seem that what I have termed the nuchal ganglion of Pœcilochætus is homologous with what he calls the hind brain in those species. But in Pœcilochætus this structure is separated sharply from the brain itself, being only connected with it by nerve-fibres. These fibres leave the brain at its posterior end, but there is no region in the brain itself which can be clearly marked off as a hind brain. The large posterior ganglion-cells to which reference has been made (Text-fig. 1, *post. gang. cells*) might at first sight be regarded as an indication of such a structure, but against this view it can be urged that they give off their processes to the region of the mid-brain, from which the commissures and tentacle nerves take origin.

The relations found in Pœcilochætus seem to indicate that it would be more correct to term what Racovitza calls the hind brain in Eurythœ and Euprosyne the nuchal ganglia. The nuchal ganglion of Pœcilochætus is clearly comparable to the palp-gaglion, and not to any division of the brain itself.

Ventral Nerve Cord—The ventral nerve-cord lies entirely in the epidermis (Pl. 10, fig. 32). The ganglia of the different segments are not very sharply marked off from each other, ganglion-cells being scattered somewhat irregularly along the whole length of the cord. Definite ganglia are, however, indicated in each segment by a considerable increase in the number of ganglion-cells, by the presence of masses of nervous felt-work (punctated substance) as well as by the roots of the lateral nerves.

Two giant fibres (fig. 32 *g. f.*) run along the cord. In preserved specimens these fibres vary in diameter in different regions, but are generally of very large size. The connection of these fibres with ganglion-cells has not been traced.

Stomatogastric System—What seems to be a well-

developed stomatogastric nervous system, comprising a ganglion and a large bundle of nerve-fibres on the pharynx, is found in Pœcilochætus, but my preparations have not sufficed to discover the complete details of its arrangement.

Lateral Sense-organs.

The position and external appearance of the lateral sense-organs are described on pp. 87—92 (figs. 1, 2, 7, etc.). In segments 1—6, as well as in the segments at the tail end of the body, it will be remembered that these organs have the form of pear-shaped papillæ protruding beyond the surface of the parapodium between the cirri, whilst in the remaining segments they lie in the parapodium with only a slightly raised rim protruding beyond the general body surface.

The histological structure is best studied in detail in organs of the latter type, as, for example, in those at about segment 20, and the most instructive general view is seen in horizontal sections of the body. Such a section is shown in fig. 34 (Pl. 10), which passes through the middle of a lateral organ. Externally the organ appears as a cup-shaped depression or crater surrounded by a raised circular rim. From the floor of the depression there springs a mass of stiff hairs, which, when the organ is not much contracted, extend far beyond the raised margin of the rim.

The external rim itself is composed of clear, transparent, epithelial cells, often showing vacuoles of some size (Pl. 10, fig. 34, *ep. r.*) These cells, as well as the face of the depression (hair-bearing area) are covered externally by a continuation of the ordinary body cuticle (*cu.*), which, excepting at the points of attachment of the muscle-fibres to be described in the next paragraph, shows no marked variation in thickness in the region of the lateral organs.

Internal Muscular System.—Immediately within the epithelial ridge the hair-bearing area is surrounded on its dorsal, its anterior and its posterior sides by bands of muscle-fibre (*m.f.*), which extend from the cuticle to the internal

apex of the organ, where they pass into the large muscles attached to that apex (fig. 34, *musc.*) The arrangement of these muscle-bands will be seen on comparing the three figures representing respectively a horizontal section (fig. 34), a section in the longitudinal-vertical plane of the animal, and therefore parallel to the hair-bearing surface of the organ (fig. 38), and a transverse section through the anterior row of muscle-bands (fig. 35). From figs. 34 and 38 especially it will be seen that along the anterior margin of the hair-bearing area a single row only of muscle-bands exists, that along the dorsal margin there are several rows, whilst on the posterior side there are two rows with a narrow strip of the hair-bearing area between them. On the ventral border there are no muscle-bands at all. The ends of the muscle-bands in contact with the cuticle broaden considerably, so that the surface of contact between the bands and the cuticle is greatly enlarged (figs. 34 and 35), the cuticle itself being at the same time very much thickened (fig. 35). The course of the fibres from the margin of the hair-bearing area to the apex of the organ is easily demonstrated in a series of horizontal sections such as fig. 34.

In fig. 35, which represents a transverse section through the anterior line of muscle-bands, it will be noticed that between the bands a row of rather large, oval nuclei exists. It is not clear to exactly what cells these nuclei belong. They may be the nuclei of the muscle-bands, in which case each band would be morphologically a single cell, or they may belong to a series of ganglion-cells of a similar type to the large ganglion-cells shown in figs. 36 and 37 (see below).

Fig. 37 is drawn from a transverse section at the level of the posterior rows of muscle-bands.

In addition to the bands already described a number of single muscle-fibres pass from the apex of the organ to the cuticle in the region posterior to the raised rim of the hair-bearing area; these are also indicated in fig. 34. All these muscle-bands and fibres stain deeply in sections.

By means of the muscular system just described, assisted by the larger muscles (*musc.*) attached to the apex of the organ, not only can the whole organ be withdrawn to a certain extent within the body, but the hair-bearing area and its rim can be at the same time still further withdrawn, until the external appearance of the organ is little more than that of a pore with a number of hairs protruding through it.

The hair-bearing cells are represented in figs. 34 and 36. The exact outlines of the individual cells are not marked out in any of the preparations, and the meaning of the appearances shown is therefore not quite clear. The great resemblance between these appearances and those shown by the ciliated cells of the nuchal groove (Pl. 11, figs. 40 and 41) and of the intestinal epithelium (Pl. 11, fig. 44) gives, however, an important clue to their interpretation.

Immediately under the external layer of cuticle is an unstained space or layer of unstained protoplasm,[1] through which the inner ends of the sensory hairs can be seen to pass just as in the ciliated cells of the nuchal organ (cf. p. 114) and of the œsophagus (p. 117).

Then follows a layer of deeply staining short rods (figs. 34 and 36 *s. r.*), which is succeeded by a layer of faintly staining long rods (*l. r.*), just as in the ciliated cells. The only difference exhibited in the two cases up to this point is that the hairs, in their course through the clear space between the cuticle and the line of short rods, stain somewhat deeply immediately below the cuticle, red in methyl-blue-eosin preparations like the short rods themselves, producing the appearance of a secondary layer of short rods (fig. 36, *s. r.* 2), which, however, is very much less marked than the main layer. This layer occupies a similar position to the layer of "bulbi" of ciliated cells, which are further referred to on p. 118.

The short rods, as in the ciliated cells, stain bright red in

[1] It is possible that the size of this space may be exaggerated by contraction of the protoplasm of the cells caused by the reagents employed. In that case the layer of short rods would lie closer to the cuticle.

methyl-blue-eosin preparations, the long rods blue. The diameter of the latter appears to be somewhat greater than the diameter of the hairs where these pass through the cuticle.

In the horizontal section shown in fig. 34, at a level immediately inside the ends of the long rods, three large oval nuclei (*n. h.*) are seen, and in neighbouring sections other nuclei appear in a corresponding position. These I take to be the nuclei of the hair-bearing cells, the interpretation being based on their similarity in situation to those of the ciliated cells of the œsophagus and nuchal organ already referred to (cf. p. 114 and p. 117). The possibility must, however, be borne in mind that these nuclei may really belong to the posterior row of muscle-bands, and their position in fig. 34 lends some support to this view. In this case they would resemble the nuclei shown in fig. 35 lying between the anterior muscle-bands. Should further investigation show this to be the case, the nuclei of the hair-bearing cells must be sought elsewhere.

Ganglion.—The remaining structure to be described in connection with the lateral sense-organ is the ganglion. The ganglion-cells may be conveniently divided into two groups—a group of large cells, which occupy the anterior dorsal portion of the organ, in front of and above the hair-bearing cells, and a group composed of a large number of small cells, which constitute the posterior portion of the organ.

The large ganglion-cells are represented in figs. 36 and 37 (transverse sections). They are large, uni-polar cells, with their processes generally directed towards the cuticle. Whether these processes eventually reach the cuticle or whether they come into contact with the hair-bearing cells I have been unable to determine with certainty. The nuclei of these ganglion-cells are very large, and either spherical or oval in shape. The contents of the cell-bodies stain deeply.

The small ganglion-cells (fig. 34, *g. l. o.*) do not for the most part show definite cell-outlines in the preparations, but appear rather as a mass of more or less closely packed nuclei,

with an intermediate substance, much of which is clearly made up of a feltwork of fine fibres. The whole structure exactly resembles what is found in parts of the brain and the ventral nerve-cord. In many sections fine fibres can be clearly seen passing from this ganglionic mass to the hair-bearing cells (fig. 34). The exact relations of these fibres to the latter cells could not be determined.

The Protruding Lateral Organs of the Anterior Segments.—Fig. 39 (Pl. 10) shows a section through one of the protruding organs of the anterior segments, the external views of which are seen in figs. 2, 3, 4, and 5 (Pl. 7). The structure is essentially the same as that of the organs already described, but the various parts are packed more closely together, so that the details are less easily made out. No further description is, however, necessary.

Connection with the Central Nervous System.—The ganglion of each lateral organ receives a bundle of fibres from a nerve which passes up the body-wall from the ganglion of the ventral nerve-cord. The course of this nerve can be easily followed in sections, its fibres lying immediately beneath the cells of the epidermis, between these cells and the muscular layers of the body-wall. After giving off the branch to the ganglion of the lateral organ the nerve continues its course in a dorsal direction, and has been definitely traced as far as the notopodial cirrus.

Muscles.—The muscles attached to the apex of the lateral organs are described on p. 125.

Historical.—The first detailed description of the structure of the lateral sense-organs of Polychætes was given by Eisig (1879 and 1887), who studied them in the Capitellids. There are some differences of importance between Eisig's account of the minute structure of the organs in Capitellids and the description of what is found in Pœcilochætus set forth in the present paper. In Capitellids Eisig describes a layer of rods immediately under the chitin, and this is followed by an irregularly arranged layer of spindle-shaped bodies. The layer of rods would seem to correspond with the long rods in

the organs of Pœcilochætus, whilst the deeply staining layer of short rods either does not exist in the Capitellids or was not rendered evident by the methods employed. The spindles of Eisig I am inclined to regard as the nuclei of the hair-bearing cells, being led to this view by a comparison of the structure of the hair-bearing cells with the ciliated cells of the nuchal grooves and of the œsophagus in Pœcilochætus. On the other hand, they may represent bipolar ganglion-cells.

Ashworth (1902) has recently written on the structure of the lateral sense-organs in Scalibregma inflatum. He describes and figures the sensory hairs, the layer of short, deeply staining rods, and the long rods, all of which have apparently the same relations as in Pœcilochætus. Ashworth, however, interprets the long rods as hair-bearing cells and the deeply staining short rods as their nuclei. With this interpretation I am unable to agree, both from the appearance of the structures themselves in my preparations as well as from a comparison with the known structure of ciliated cells (cf. p. 118).

Ashworth also describes and figures large unipolar and bipolar ganglion-cells similar to those found in Pœcilochætus, and states that the processes of these cells can be traced into continuity with the internal ends of the rods which carry the sense-hairs. I have been unable to make out with certainty such a connection in Pœcilochætus, though the appearances presented are in no way opposed to its existence.

Further studies on the lateral sense-organs in the different groups of Polychætes, made with the aid of more special methods for determining the course of the nervous fibres, are necessary before their structure can be fully understood.

Nuchal Organ.

One of the most characteristic features of the genus Pœcilochætus is the great development of the nuchal organ, which, as already stated, consists of a broad, basal portion springing from the dorsal surface of the posterior end of the

head, and of three long, tentacle-like processes extending backwards from it (fig. 7, *nuch.*). Of these three processes, the middle one is the longest, and may run at least as far backwards as segment 6, the lateral ones ending about segment 4 (fig. 1). The lateral processes have occasionally been observed with a secondary branch. The whole organ is covered with a number of sensory hairs, and each process possesses two lateral ciliated grooves, which run along the whole of its length and extend on to the basal portion (Pl. 11, figs. 42 and 47, *nuch.*).

Claparède (in Ehlers, 1874) and Levinsen (1883) have both described the three processes, but have failed to recognise their true nature as nuchal organs. These two authors have, however, shown clearly that the organ in question develops as an outgrowth from the posterior cephalic region. Such enlarged nuchal organs are by no means unknown amongst Polychætes, though none have yet been described having dimensions comparable with those of Pœcilochætus. The nuchal organs of Virchowia clavata figured by Viguier (1886) may be referred to, as well as those of Amblyosyllis spectabilis and Autolytus longiferiens, figured by Malaquin (1893). Gravier (1896) describes the nuchal organ of Notophyllum, which takes the form of two lappets extending from the posterior end of the prostomium to the middle of the third segment. Racovitza (1896) shows that the caruncle of the Amphinomidæ is an enlarged nuchal organ.

In the living Pœcilochætus the nuchal organ has a brown or brownish-green colour. Sections show that this colour is due to granules deposited in the epidermic cells, and also to a number of spherical bodies scattered through the tissue, which possess a single, deeply staining nucleus, and are filled with dark granules (Pl. 11, fig. 40).

The base of the nuchal organ is occupied by the nuchal ganglion (fig. 42, *nuch. gang.*), which has already been described (p. 104). The central axis of each of the processes of the organ is formed by a tube lined with mesoderm-cells, the tube being in direct communication with the general

body-cavity of the first segment of the worm. The space between this central canal and the epidermis is filled with an irregular mass of cells, forming a loose tissue, in which may be seen many of the spherical bodies filled with granules mentioned in the last paragraph. Figs. 40 and 41 (Pl. 11), representing respectively a transverse section through one of the ciliated grooves and an enlarged section of a portion of the epithelium of a groove, show the minute structure of the tissue of the nuchal organ. The epidermis, excepting in the ciliated grooves themselves, consists of low epithelial cells crowded with dark-coloured granules, the granules being in many places congregated into masses of considerable size. The epithelial cells are covered externally by a layer of cuticle resembling the general body cuticle. No gland-cells, such as have been described in other parts of the body, have been observed in the nuchal organ.

The ciliated epithelial cells of the grooves are very large and much elongated, the protoplasm of the bodies of the cells is filled with dark granules similar to those found elsewhere in the organ, and the large oval nuclei lie near the bases of the cells, their long axes being parallel to the long axes of the cells. The structure of the external or ciliated ends of the cells presents features of interest, and can be seen from figs. 40 and 41. The cuticle (*cu. g.*) covering the cells undergoes a very considerable external thickening. In sections stained with methyl-blue-eosin the basal portion only of this cuticle stains a deep blue, carrying on the line of the general cuticle of the nuchal organ; the external thickened portion remains clear and unstained (*cu. g.*), and in favourable places is seen to be traversed by a series of faint lines running at right angles to its surface. Since these lines are much more widely separated than the cilia, they would seem not to be due to the cilia passing through the cuticle. Racovitza (1896) has described a very similar thickening of the cuticle in the caruncle of Euphrosyne Audouini. In the case of that worm, however, the thickening does not extend over the areas of cuticle lying just above the ciliated cells.

Immediately inside the cuticle is a narrow zone, which in sections appears clear, but across which the cilia can be seen to pass. This zone may, to some extent at least, be due to shrinkage of the bodies of the cells during preservation and their consequent withdrawal from the cuticle. It is followed by what, in transverse sections, appears as a deeply staining line (stained red in methyl-blue-eosin preparations). This line, on examination with high powers, resolves itself into a layer of deeply staining short rods (fig. 41, *sr.*), one rod apparently corresponding with each cilium. Within this layer of deeply staining rods the internal ends of the cilia can be followed for a considerable distance as faintly staining rods (blue in methyl-blue-eosin preparations), the diameters of which appear somewhat greater than the diameters of the cilia outside the body (fig. 41, *l. r.*). In the portion of the cell occupied by these rods the protoplasm of the cell appears clear, and not granular as it does throughout the general body of the cell. These relations will be seen to correspond with those found in the ciliated cells of the wall of the œsophagus (p. 117, *et seq.*, where the work of previous authors is discussed, Pl. 11, fig. 44) and in the hair-bearing cells of the lateral organs (p. 108, Pl. 10, figs. 34, 36, 39).

In the nuchal organ of Pœcilochætus, I have failed to identify nerve-cells, other than the ganglion-cells already described in the basal portion of the organ. It is quite possible, however, that some of the cells of the intermediate tissue are really such nerve-cells.

For a full historical account of the nuchal organ in Polychæta, as well as for an excellent description of the detailed histological structure of that organ in a number of different types, reference should be made to the paper by Racovitza (1896) already several times mentioned.

Eyes.

As previously stated, Pœcilochætus possesses four eyes, one pair on the dorsal surface of the prostomium, and a

larger pair on the ventral surface. All four eyes have practically the same structure, and are of a very simple type. Fig. 33 (Pl. 10) represents a section through one of the ventral eyes cut in the longitudinal vertical plane. The eye consists of a single large optic cell with one nucleus. The protoplasm of the swollen, rounded end of the cell is slightly modified, being more transparent than that found in the rest of the cell, and showing indications of a radial structure. This end of the cell is surrounded by a large cup-shaped mass of black pigment, made up of numerous spherical drops of black substance. A nucleus can often be detected at the outer margin of this mass of pigment, but I have been unable to satisfy myself as to whether more than one nucleus belongs to each; that is to say, whether the pigment cup is unicellular, or whether it consists of several cells.

Such simple eyes are now well known amongst the Platyhelminths, for instance, in Planaria torva, and also in certain Polychætes, as, for example, Spio fuliginosus and Polyophthalmus pictus. A full account of the literature of the subject, together with a wealth of new observations, will be found in the series of papers "Untersuchungen über die Organe der Lichtempfindung bei niederen Thieren," by Richard Hesse (see especially Hesse, 1897 and 1899).

Alimentary Canal.

The Divisions of the Alimentary Canal.—The external features of the mouth are seen in fig. 8 (Pl. 8), and have already been described (see p. 88). The animal possesses a short proboscis with thick walls, which in preserved specimens is always almost if not entirely withdrawn into the mouth. Fig. 42 (Pl. 11) represents a section through these parts. The external folds surrounding the mouth are seen, as well as the median tentacle (*m. tent.*), which has its point of insertion close to the anterior edge of the mouth, into which its basal portion can be withdrawn.

The portion of the alimentary canal extending from the

mouth to the posterior septum of the eighth segment may be conveniently divided into two parts corresponding to what are known in other Polychaetes as œsophagus and pharynx (or gizzard). There is, however, no definite line of demarcation between these two parts. The œsophagus is lined by elongated, ciliated epithelial cells, outside which is a thin layer of annular muscles followed by a thin layer of longitudinal ones. Proceeding further backwards the epithelial layer becomes narrower, the cells being considerably less elongated, whilst, on the other hand, the muscular layers, especially the layer of annular muscles, become much more strongly developed (cf. figs. 42 and 48 with fig. 43). It is this muscular portion which may be termed the pharynx (*ph.*) In its hinder part the epithelium is thrown into folds or villi, and at the point where the septum of segment 8 is attached to the alimentary canal one large fold, forming a kind of valve (fig. 43, *v.*) seems to constitute a definite line of demarcation between the pharynx and the intestine. As is explained on p. 123, the septa in this region of the body are pushed very much backwards; the junction of the pharynx and intestine, although really at the posterior end of the eighth segment, may lie as far back as the level of the twelfth parapodia. From the posterior septum of segment 8 to the posterior septum of segment 13, the intestine continues as a comparatively straight tube, not differing much in structure from the pharynx, excepting that the muscular layers are rapidly reduced until they almost entirely disappear (figs. 43 and 50). In segments 14 and 15 the intestine is considerably dilated, but narrows again as it passes through each septum. In the segments from 16 backwards, this enlargement of the intestine in each segment becomes very great, so that the dilated intestine occupies a large part of the body-cavity (figs. 1 and 58). In the living worm these intestinal pouches are constantly expanding and contracting, the movements of the intestine constituting, in the posterior region of the body, the principal mode of circulation of the blood.

Ciliated Groove of the Alimentary Canal.—Transverse sections show the existence of a deep, longitudinal ciliated groove running along the mid-ventral line of the alimentary canal throughout its entire length. A similar groove has been described in many Polychætes, and is homologous with the secondary intestine ("nebendarm") of the Capitellids (Eisig, 1887).

The Epithelium of the Alimentary Canal.—The epithelium of the alimentary canal, though differing much in appearance in its different parts, consists essentially of cells of two kinds, (1) columnar epithelial cells, and (2) goblet-shaped gland-cells lying between the columnar cells and opening by more or less narrow necks into the lumen of the canal.

The columnar epithelial cells of the œsophagus (Pl. 11, fig. 44) are narrow and elongated, with large, oval nuclei, the long axes of which lie parallel to the long axes of the cells. The cells themselves are strongly ciliated and show a characteristic structure, similar to that which exists in other ciliated cells of the worm (compare the groove of the nuchal organ, p. 114, the lateral sense-organs, p. 108, and the epithelium of the genital funnels, p. 135). The appearance presented by the ciliated borders is seen in fig. 44. The surface of each cell is covered by a thin cuticle (*cu.*) continuous with the cuticle of the external body surface. Immediately within this cuticle is a clear space through which the inner ends of the cilia are seen to pass. This clear space, which may in part at any rate be due to contraction of the contents of the cells during the preservation of the tissue, is bounded internally by a layer of deeply staining short rods (*s. r.*), which appear to be in reality slightly thickened, deeply staining portions of the individual cilia. In sections these rods lie in a straight line which runs parallel to the cuticle. Beyond this deeply staining layer of rods the ends of the cilia (*l. r.*) can still be traced for some little distance into the protoplasm of the cell-body, which is at first clear excepting for the striation due to these inner ends of the cilia, and subsequently

becomes granular, many of the granules being of a characteristic yellowish-brown colour. The cilia cannot be traced as far as the nuclei of the cells. In sections of material fixed in Hermann's fluid and stained with a mixture of methyl-blue-eosin (fig. 44), the cuticle stains deep blue, the cilia faintly blue, the layer of short rods bright red, the cell protoplasm bluish, whilst the nuclei are clear, with chromatin granules and network stained deep red.

The structure of the ciliated cells above described, as well as those of the nuchal groove (p. 114) and the hair-bearing cells of the lateral sense-organs (p. 108), agrees with that found by Engelmann (1880) in the ciliated cells from the intestine and gills of Cyclas cornea and Anodonta, and in the ciliated cells from the nose of the frog. Engelmann clearly describes the short rods under the name of "Fussstücke," and also the internal prolongations of the cilia within the cells, which he was, however, able to trace much further into the body of the cell than I have succeeded in doing. He also found between the short rods and the cilia proper a certain differentiation of the substance of the bases of the cilia, which he calls the "bulbus." These "bulbi" would appear to correspond to the secondary layer of short rods described in the present paper at the bases of the sensory hairs of the lateral sense-organs (p. 108, Pl. 10, figs. 34, 36, 39).

Englemann states that Eimer was the first to describe correctly the relations of the short rods (Fussstücke) to the cilia, and he gives several other references to previous papers dealing with the subject.

More recently Greenwood (1892) has shown a very similar structure in the ciliated cells of the intestine of Lumbricus, though in the latter worm, to judge by the figures, the layer of short, deeply staining rods is less marked. Greenwood, however, states (p. 245) "the cilia occasionally bear tiny varicosities before they pass into the body of the cell. Under a sufficiently high power these are distinguishable as belonging each to a ciliary thread, and they recall Heidenhain's description of similar thickenings, which may be seen

under suitable conditions at the base of the intestinal rods of the dog."

The similarity in structure of the ciliated cells of, say, the œsophagus and nuchal organ of Pœcilochætus to that of the cells of the intestinal mucous membrane of vertebrates furnished with a "striated border," as described by Heidenhain (1888), is very striking, especially if his fig. vi is compared with my figs. 44 or 41, and seems strongly to support the suggestion contained in Greenwood's paper, that the cells of the vertebrate intestine may be modified ciliated cells.

Galvagni (1903) has just published a description with figures showing a similar structure in the ciliated cells of the alimentary canal of Ctenodrilus to that found in Pœcilochætus.[1]

In concluding the discussion on these ciliated cells it seems worth while to draw attention to the possibility that the "short rods" described in this paper ("Fussstücke" of Engelmann) are homologous with the middle-piece ("Mittelstück") so well known in spermatozoa. The staining reactions of the two structures are similar, and they occupy similar positions in relation to the cilium and flagellum respectively.

The goblet-shaped gland-cells in the epithelium of the œsophagus present themselves in at least three forms. In preparations preserved and stained by my usual method these cells show the following features (see Pl. 11, fig. 44) :—(1) Goblet-shaped cells crowded with granules which stain bright red, the bright red granules filling both the body of the cell and the long neck to where it opens into the lumen of the œsophagus; the intermediate substance of the cell remains unstained; the chromatin of the nucleus stains red; the nuclear membrane and the body of the nucleus are clear and unstained (fig. 44, *gl.* 1).

(2) Goblet-shaped cells containing granules, which are less

[1] Since the above was written an important paper on the epithelium of the intestine of Polychætes has been published by Brasil (1904) in which the structures here described are fully dealt with.

numerous than those in cells of the previous type and stain blue instead of red (fig. 44, *gl.* 2).

(3) Cells still of the same general shape, but less swollen, without granules, but filled with a homogeneous substance staining faintly blue and showing at most slight indications of a network such as is usually produced by the action of preserving fluids; the nuclei of these cells stain more deeply and more diffusely than those of the previous types, their ground substance taking on a faint blue tint, whilst the chromatin is red or purple (fig. 44, *gl.* 3).

Cells of the first and second kinds are clearly actively secreting cells, whilst those of the third kind seem to be cells of the second which have completely discharged their secretion and are in a resting condition, in all probability waiting to commence the secretory process upon suitable stimulation. In some specimens nearly all the gland-cells in the œsophagus are in the condition last described.

As will be seen by comparing the two sets of figures and the two descriptions, the gland-cells of the œsophagus show many points of resemblance with the gland-cells of the skin and of the parapodial cirri.

The structure of the epithelium of the pharynx and of the anterior portion of the intestine is essentially the same as that which has been described for the œsophagus; the cells, however, become gradually less elongated in shape, and the number of gland-cells diminishes.

At about segment 16 or 17 the type of intestinal epithelium which persists through the greater part of the body of the worm is established. This epithelium is found in two markedly different conditions, which appear to depend upon whether the intestine is filled with food and digestion is actively going on, or whether food is absent from it. These two conditions are illustrated in figs. 45 and 46 (Pl. 11).

Fig. 45 shows the state of things which is found when food is present and digestion is actively proceeding. The epithelial cells (*ep.*) are large and swollen, whilst the gland-cells have shrivelled till little more than the nucleus is visible (*gl.*).

The shape of the epithelial cells may differ considerably from that of those found in the anterior part of the alimentary canal already described. In those shown in fig. 45 the cell-body is short and broad, but more elongated cells are also common. The cells are filled with large granules, which have a dark brown colour in preparations preserved with osmic acid mixtures. The granules are crowded together at that surface of the cell which immediately borders the lumen of the intestine, and are more scattered throughout the rest of the cell protoplasm. These cells have not, in my preparations, the appearance of being ciliated. Their surface is, however, covered with a faintly staining substance, which might possibly represent broken-down cilia, but is more probably a layer of the food-contents of the intestine, which is being absorbed by the cells. In the same sections the cilia are often sufficiently well-marked on the cells of the intestinal groove. The nucleus is situated near the base of the cell. In the condition now being described (fig. 45) it is large in size and stains deeply (diffuse blue with red granules in methyl-blue-eosin preparations). It consists of an outer membrane filled with granules, and possesses a single nucleolus. This nucleolus is surrounded by a clear space, the space being bordered by a membrane which carries on its outer side a deeply staining, hemispherical cap. The nucleus thus resembles very closely the nuclei of the parapodial gland-cells already described (cf. fig. 26 and p. 98). Nuclei showing clearly all the points mentioned are not, however, met with very frequently in the preparations. The bases of these cells lie close to the blood-sinus which completely surrounds the intestine (fig. 45, *i.bl.s.*).

If one may be permitted to hazard a guess at the physiological processes which are going on in these cells, merely from a study of their appearance and the arrangement of their parts, it may be suggested that the cells at their free ends are absorbing from the cavity of the intestine food material already partly digested by the action of the secretion from the gland-cells. A portion, at least, of this material

appears in the cells in the form of the yellowish-brown granules, which are thickly congregated at the surface of absorption. The nucleus is obviously in a very active state, and its position at the base of the cell, at the point of contact of the cell with the intestinal blood-sinus, seems, if we accept the view advocated by Korschelt that the nucleus is generally to be found where the chief function of the cell is in active progress, to suggest that the food substance there undergoes transformation and is passed through the cell-wall into the blood.

The second condition in which the epithelial cells of the intestine are found (when the intestine does not contain food) is illustrated in fig. 46. The gland-cells (*gl.*), which in the former state were shrivelled and inert, are now large and active. They are pear-shaped, filled with granules (which in methyl-blue-eosin preparations stain bright red), and their necks extend quite to the surface of the epithelial layer. The nuclei are clear and transparent, with deeply staining chromatin, the greater part of which is concentrated in a single large nucleolus.

The columnar cells (*ep.*), on the other hand, contain no granules; their protoplasm stains faintly and diffusely (blue in methyl-blue-eosin preparations), and shows only an indefinite reticulation probably due to the action of the reagents. The nuclei are much smaller than in the active epithelial cells previously described (cf. figs. 45 and 46), the chromatin granules stain less deeply (red), and the whole nucleus is diffusely tinted (blue). It would seem, therefore, that whilst the gland-cells are now active the columnar cells are inert.

The epithelium in only two other parts of the alimentary canal calls for mention, namely, that in the ventral ciliated groove and that in the terminal segments of the body.

The cells of the ciliated groove are elongated and distinctly ciliated, though they do not show the layer of deeply staining short rods, which was found in the ciliated cells of the œsophagus.

The epithelium of the intestine in the posterior region of the body (rectum) differs only from that already described for the intestine in the fact that all the cells are ciliated, the cilia being very long. The action of these cilia can be clearly seen in the living worm.

Body-cavity.

The well-marked segmentation of the body seen externally is equally distinct internally, each segment being separated from that which follows it by a transverse septum. The septa, the first of which lies between the first and second segments, appear to divide the body-cavity into a number of separate compartments, between which no communication can be shown to exist. These compartments are not, however, of equal size, for in the region occupied by the muscular pharynx the septa, instead of lying in a vertical plane corresponding to that of the external segmentation of the body, are pushed backwards for a considerable distance. This pushing backwards of the septa, which is shown in the sagittal section represented in fig. 47 (Pl. 11) and in the horizontal section fig. 43, commences with the septum at the posterior end of segment 5, reaches a maximum in segment 8, and is still obvious in segment 12. The posterior septa of segments 8, 9, 10, and 11 all extend back to the region, which external segmentation indicates as segment 12, and that of segment 12 is pushed back into 13. The septa of segment 8 to 11 join the alimentary canal immediately behind the point where the pharynx passes into the intestine (fig. 43, *v.*). These septa are also noteworthy from the fact that the muscle-fibres, which are present to a considerable extent in the septa of most of the segments of the body, are here developed to a very remarkable extent, so that septa 8 to 11 have become highly muscular organs. This muscular character of the septa, combined with the manner in which they are pushed backwards, seems to suggest that they are concerned with the protrusion of the anterior portion of the alimentary canal in the form of a proboscis, and probably

also with the extension of the palps. I have only once seen a protrusion of the proboscis, and it did not then extend much beyond the front of the head. The structures just described, however, appear to suggest the possibility of a much greater protrusion.

In living specimens of Pœcilochætus an indication of the backward extension of the septa of segments 5 to 12 can be seen in the backward course of the lateral blood-vessels, which run in the septa (fig. 1 and p. 126).

In the segments from the thirteenth backwards, the internal and external segmentation correspond.

Each of the septa dividing the body-cavity consists of a double layer of cœlomic epithelial cells with a layer, more or less strongly developed, of muscle-fibres between. The epithelium is extended over the main body muscles, and over the other organs of the body. On most organs, however, the cells are seldom much developed, their presence being often only indicated by occasional nuclei.

Extensions of the body-cavity into the interior of the nuchal organ and of the palps are mentioned in the paragraphs dealing with those structures.

Musculature.

The muscles of the body-wall, as is usual in the Polychætes, are arranged in two layers, a layer of annular muscles and a layer of longitudinal. Of these two layers, however, the annular is very feebly developed in Pœcilochætus, whilst the longitudinal is well developed. The principal muscles in each segment are massed into four bundles, two dorsal lying on either side of the dorsal blood-vessel and two ventral on either side of the nerve-cord. The slight development of the annular muscles would appear to be connected with the more or less sedentary habits of the worm. The annular muscles attain their greatest development in worms which burrow constantly and rapidly in the soil (e. g. Nephthys, Aricia).

Bands of oblique muscles run from the outer dorsal edge of the ventral nerve-cord on each side, pass over the longitudinal ventral muscle-bands, and are inserted in the lateral walls of the body between the parapodia.

External Muscles of the Lateral Organs and Muscles of the Chætal Sacs.—Four large bands of muscle are inserted at the apex of each lateral organ (Pl. 10, figs. 34 and 37, *musc.*), viz. (1) a band which runs downwards and inwards to the inner end of the neuropodial chætal sac, which is clearly one of the two bands used to protrude the chætæ; (2) a similar band running upwards and inwards to the base of the notopodial chætal sac; (3) a broad band of muscle which runs from the lateral organ downwards, passes behind the chætal sac and is inserted in the ventral body-wall below the base of the neuropodial cirrus, and (4) a similar band running upwards and inserted in the dorsal body-wall above the base of the notopodial cirrus. Lying in contact with the muscle-fibres of bands 3 and 4 are a number of fibres, which run direct from the dorsal to the ventral body-wall. These pass close to the apex of the lateral organ, to which they are joined by connective tissue, but they have no free ends inserted in that apex.

In addition to the muscle-bands described above, (1) and (2), running from the apex of the lateral organs to the inner ends of the two chætal sacs, a second band runs from the inner end of each sac, passes in the one case downwards and outwards and in the other upwards and outwards and is inserted in the body-wall. Thus each sac has two strong muscles from its apex to the body-wall, one above and one below, by the contraction of which it and its chætæ are protruded.

Blood System.

The anatomy of the vascular system constitutes one of the most striking and interesting features of Pœcilochætus. The bright scarlet of the blood gives to the anterior portion of the body its characteristic colour, and the

alternate filling and emptying of the larger vessels produces an appearance of rapid colour-change. A further change of colour is seen also, which is due to changes in the chemical character of the blood. If a worm be allowed to remain for some time in a vessel containing only a small quantity of sea-water, the bright scarlet of the blood changes to a dull purple-red, but the original colour immediately reappears on the addition of a new supply of water, as it does under similar circumstances in Magelona (cf. Benham, 1896). The red colour therefore would seem to be due to the presence in the blood of one of the respiratory pigments.

The general arrangements of the principal vessels is illustrated in fig. 1, which has been constructed from observations on the living worm, corrected and extended by the examination of sections. For the purposes of description the body of the worm must be divided into three regions, an anterior region consisting of segments 1 to 11, an intermediate region, comprising the four segments 12, 13, 14, and 15, and a posterior region from segment 16 to the end of the body.

Anterior Region.—Between the alimentary canal and the dorsal body-wall there is in the anterior region a large, muscular, dorsal vessel of cylindrical shape capable of very considerable expansion, waves of expansion and contraction passing along it from behind forwards.

Corresponding with each of the body segments from the third to the eleventh, a lateral vessel is given off on each side from the dorsal vessel, and runs outwards and downwards in the posterior septum of the segment. Owing to the fact already described that the posterior septa of segments 5 to 11 are pushed backwards, the origins of the lateral vessels in these segments, running, as the vessels do during the first part of their course, actually in the septum, are also carried backwards, the vessels being in consequence much elongated and running forwards (Pl. 7, fig. 1). In this way the lateral vessels belonging to segment 7 arise from the dorsal vessel at about the plane of the junction of the ninth and tenth para-

podia, whilst the lateral vessels of segments 8, 9, 10 and 11 arise close together about the level of the twelfth parapodia.

On reaching the base of the parapodium of the segment to which it belongs, the lateral blood-vessel in each case leaves the septum and sends a loop forwards into the parapodium, the loop returning upon itself and joining the septum again in the neighbourhood of the internal opening of the nephridium. The vessel here divides into two branches. One of these branches passes downwards and inwards and opens directly into the large longitudinal ventral blood-vessel, the other passes through the septum close to the tube of the nephridium, and in the segment behind divides up into a number of blind, finger-shaped processes, which spread out in the body-cavity of that segment. Under favourable circumstances these finger-shaped processes can be seen in the living worm, alternately expanding and contracting as they fill with the bright red blood and empty themselves again. On one occasion, in a worm the body-wall of which had burst on compression, I was fortunate enough to see one of these clusters of finger-shaped vessels lying outside the body and to satisfy myself that each process visible ended blindly. The vessels when filled with blood are very conspicuous, and easily followed in sections (Pl. 11, figs. 48 and 49), and in spite of repeated attempts I have never been able to find that this cluster of vessels has any communication with the rest of the blood system, excepting through the branch of the lateral vessel of the segment in front, which accompanies the nephridial tube through the septum dividing the two segments. Fig. 51 (Pl. 12), drawn from a longitudinal vertical section, shows clearly the branch of the lateral vessel (*b. lat. v.*) passing back through the septum into the segment behind and there breaking up into finger-shaped processes (*f. p.*). Fig. 49 (Pl. 11) represents a transverse section through the finger-shaped processes (*f. p.*), and shows the great enlargement of the blood-vessel which can take place at the point where the processes are given off. Fig. 48 (Pl. 11) shows well the latter part of the course of the lateral vessels (*lat. v.*) to the ventral vessel.

Shortly after leaving the dorsal vessel, each lateral vessel gives off a branch, which breaks up upon the wall of the œsophagus and pharynx, uniting with and helping to form a rich network of blood-vessels, which extends over the surface of these organs (Pl. 7, fig. 1). This network also gives rise to vessels which start from the under surface of the œsophagus and pharynx and pass directly downwards to the ventral vessel (fig. 48, *int. v.*) Blood can thus pass either directly from the dorsal to the ventral vessel through the laterals, or indirectly after passing through the network on the walls of the alimentary canal.

At its anterior end, immediately behind the brain, the dorsal vessel bifurcates (Pl. 7, fig. 1), sending a large vessel to each of the palps. These large vessels pass along the axes of the palps (Pl. 10, fig. 30), and in the living worm are subject to rhythmical pulsations, which keep the blood within them constantly in motion. The palps appear to be one of the principal organs of respiration of the worm (see p. 86).

Immediately after entering the palp the large blood-vessel gives off a branch, which passes downwards and backwards through the first and second segments. It sends one secondary branch to the œsophageal network and another through the posterior septum of the second segment to form a cluster of blind, finger-shaped vessels in the third segment, and then, passing below the œsophagus, joins with its fellow of the opposite side to form the anterior end of the ventral vessel. These structures can best be understood from an examination of fig. 1.

As only one blood-vessel passes along the axis of each palp, it would seem that the pulsations of the vessel itself must take place in such a manner as alternately to drive blood in and then out of the vessel, but owing to the readiness with which the palps are thrown off on the slightest irritation, direct observations on the point are not easy to make.

The Middle Region.—The modification of the vascular system in segments 12, 13, 14 and 15, the middle region of the body, is of special and peculiar interest.

In each of these segments the dorsal vessel is itself much

enlarged, forming on either side large lateral pouches, which are alternately inflated with blood and emptied (Pl. 7, fig. 1; Pl. 11, figs. 43 and 47, *p. dv.*). When fully inflated the pouches occupy almost the whole of the body-cavity, and the wave of expansion and contraction, passing from segment to segment from behind forward, is a striking phenomenon.

The forward movement of the blood from one segment to the next in front is regulated by a series of valves situated in the dorsal vessel between each successive pair of pouches, as well as immediately anterior to the first pair and posterior to the last. There are thus five valves altogether. These valves, two of which are shown in sagittal section in fig. 50 (Pl. 11), consist of somewhat stout membranes composed of spindle-shaped cells, attached ventrally to the wall of the blood-vessel, but with a free dorsal edge, which pressed from in front comes into contact with the wall of the vessel and prevents the blood from passing backwards. In fig. 50 the anterior valve (*vl. seg.* 14) is open, whilst the posterior valve (*vl. seg.* 15) is closed. It is clear that contraction of the walls of the blood-vessel and its lateral pouches will force the blood forwards, whilst the valves will prevent any blood from going in the opposite direction.

It must be pointed out that the lateral pouches of the dorsal vessel in segments 12, 13, 14 and 15 are not swollen lateral vessels, such as are described by Benham (1896) in Magelona, for in sections the true lateral vessels, similar in their general relations to those of the anterior segments, and like them giving rise to a cluster of finger-shaped processes in the segment behind, are easily seen and followed. These lateral vessels arise from the dorsal vessel in each case behind the lateral pouches, at the point where the dorsal vessel passes through the posterior septum of the segment, and they run throughout the greater part of their course in this septum.

The Posterior Region.—In the posterior region of the body, from segment 16 backwards, the arrangement of the vascular system undergoes a great change. The dorsal vessel can no longer be distinguished as a separate organ, but the

dorsal vessel and the network of blood-vessels which surrounded the œsophagus and pharynx, have as it were run together to form one large sinus, which completely surrounds the intestine (figs. 45 and 59, *i. bl. s.*). The circulation of the blood is now brought about, not by the contraction of a blood-vessel with highly muscular walls, but by the contraction and expansion of the segmental pouches of the intestine.

The intestinal sinus communicates with the ventral vessel by short, vertical branches, and it also gives off in each segment, from the region of the narrowed portion of the intestine behind the intestinal pouches, the lateral blood-vessels. Each lateral blood-vessel runs in the posterior septum of the segment, at first upwards and outwards, then outwards and downwards, to the base of the gills on the notopodium. It enters the first gill filament, to the tip of which it runs; it there turns sharply on itself and comes back again to the base of the gill, thus forming a single, simple loop, which occupies the whole of the interior of the gill filaments (Pl. 10, fig. 31). After having formed a similar loop in all the other gill filaments the blood-vessel again runs in the posterior septum of the segment, its course being inwards and downwards to the ventral vessel, which it joins. During this latter part of its course the vessel sends back a branch along the tube of the nephridium into the segment behind, which appears to supply not only the nephridium, but also the genital organs, which lie along the tube of the nephridium (Pl. 12, figs. 52 and 60, *b. lat. v.*). There is, however, no obvious formation of a cluster of blind, finger-shaped processes such as was met with in the anterior segments of the worm.

One point of interest in connection with the lateral vessels remains to be noticed. In describing the course of the lateral vessels of the anterior segments, it was stated that each vessel, before sending back its branch to the finger-shaped processes in the segment behind, ran forwards and formed a simple loop at the base of the parapodium. It will be remembered that the parapodia of these anterior segments

have no gills, but the loop just mentioned would seem to represent in a rudimentary way the loops of the lateral vessels which supply the gill filaments in the posterior gill-bearing segments.

The Structure of the Walls of the Blood-vessels.—The different layers of the walls of the blood-vessels attain their greatest development in the dorsal vessel. This vessel is lined internally by an epithelial layer consisting of flattened cells, the general height of which is less than the diameter of their nuclei, so that the portion of the cell in the immediate neighbourhood of a nucleus often appears to protrude into the blood-space. The bodies of the cells generally remain clear and unstained.

Proceeding outwards from this epithelial layer, one finds a layer of longitudinal muscle-fibres, which is followed by several layers of annular muscles, this part of the wall being especially developed in the dorsal vessel. The whole vessel is covered externally by a layer of cœlomic epithelial cells, which form the lining of the body-cavity. Like that of the cells lining the vessel internally, the protoplasm of these external cells remains clear and unstained (Pl. 11, fig. 50).

The differences met with in the structure of the walls of the other blood-vessels of the body are due to the reduction of the various layers, more especially of the muscular layers. In the ventral vessel, as well as in the lateral pouches of the dorsal vessel, the epithelial layers are well developed, but the muscular layers, though still obvious, are greatly reduced. In the lateral vessels and their various branches, especially when extended with blood, only a thin membrane in which an occasional nucleus is seen can generally be recognised. It is probable, however, that both epithelial layers are present, whilst the muscular layer has almost, if not entirely, disappeared.

In the external wall of the intestinal blood-sinus the two epithelial layers can be made out, with a layer of muscle-fibres between them. Internally the bases of the intestinal epithelial cells appear to be separated from the blood-space by

a thin layer of very flat cells, but the existence of this layer is not easy to demonstrate satisfactorily. Strands of tissue cross the blood-space at intervals, having the appearance of prolongations of the epithelial-lining-cells.

It should be noted that in the walls of the intestinal pouches no muscle-fibres can be demonstrated excepting those in the outer wall of the blood-sinus, and the contractions of the pouches would seem to be brought about by these muscles (figs. 45 and 46).

The Blood.—The blood of Pœcilochætus is a bright scarlet coloured, homogeneous fluid without corpuscles of any kind. Very occasionally in sections an isolated cell, having a similar appearance to the cells of the epithelial lining of the blood-vessels, is seen in the blood-space. Such cells are probably only cells of this epithelium which have become detached.

The change of colour of the blood caused by want of oxygen has already been described (see p. 126).

Nephridia and Nephromixia.

In small living examples of Pœcilochætus viewed from the ventral surface the nephridial organs can be seen as short greenish-brown tubes, one pair in each segment, commencing at the level of the anterior septum, running first backwards and then turning outwards and forwards, and ending on the antero-ventral face of the parapodium. By examining the more transparent segments near the tail end of the worm with a moderately high power it can be further seen that anteriorly the nephridium opens in a large ciliated funnel, and that the whole length of the tube from the anterior internal opening to the external opening at the base of the parapodium is strongly ciliated.

The details of the structure of these organs can be made out with some clearness in series of sections of specimens preserved in Hermann's fluid, more especially in longitudinal vertical (sagittal) and in horizontal sections of the worm.

Adopting the nomenclature of Goodrich (1900, p. 742), it

may be stated that both nephridia and nephromixia are found in Pœcilochætus. Nephridia, opening by nephridiostomes into the next segment in front, are found in the anterior segments (4 to 16), whilst compound organs (nephromixia), consisting of nephridia with large genital funnels (gonostomes) attached to the nephridiostomes, are found in the genital segments from segment 17 backwards.

In the two anterior body segments (1 and 2) no trace of a nephridium has been detected. In segment 3 the nephridiostomes of the organs of the following segment are well developed, and they, as well as the organs to which they belong, have the structure about to be described, which is typical of that in all the segments from 4 to 16. The nephridial tubes in each of these segments are simple and J-shaped (cf. fig. 58), running from the anterior septum of the segment straight backwards, then turning outwards and forwards to the external opening on the parapodium, as already described. The cells lining the tubes are low, elongated, ciliated cells, which contain large numbers of excretory granules. The lips of the nephridiostomes, which lie close to the posterior septum of the segment next in front, form a structure of considerable size, with a small ciliated aperture which puts the lumen of the nephridial canal into communication with the body-cavity of the latter segment. Fig. 55 (Pl. 12) represents a transverse section through a nephridiostome of one of these segments, and fig. 54 a sagittal section. The lips (*lp.nst.*) form masses of swollen cells filled with vacuoles and granules. These masses of cells are attached to the posterior septum of the segment along a somewhat narrow border, and protrude for some little distance into the cavity of the segment (cf. fig. 47). The nephridiostome itself (*nst.*) lies near the lateral wall of the body, but the swollen masses of cells forming its lips run inwards almost to the median line of the body. This inward extension of the lips is seen in the transverse section (fig. 55), and is also well shown in horizontal sections. Externally the lips are covered by a layer of cœlomic epithelium (fig. 54).

Fig. 57 (Pl. 12) shows the appearance presented by cells of the nephridial lip under a high power, the figure being drawn from a section of material preserved in Hermann's fluid and stained with methyl-blue-eosin solution. The cells are much swollen and vacuolated, and contain, in addition to the nuclei (*n.*), large numbers of granules of various sizes, which stain bright red in the preparations. The protoplasmic ground substance of the cells stains blue, but the cells, being highly vacuolated, this blue-staining substance is not uniformly distributed through them. The red granules are often surrounded by a spherical mass of blue-staining protoplasm, in the case of the smaller granules two or three being found within each sphere. The appearances suggest that the red granules are first formed within the blue spherical masses, that they gradually increase in size within these masses, whilst the latter subsequently become swollen and break down, giving rise to the vacuolated appearance of the general cell protoplasm with free red granules floating in it.

In the segments from 17 backwards the structure and general shape of the nephridia themselves (fig. 58) remain practically the same as in the anterior segments, excepting for the fact that a large ciliated genital funnel is added to the nephridiostome. The arrangement of this genital funnel will be gathered from the sagittal section shown in fig. 52 (Pl. 12). The upper portion of the funnel is formed by a great development of the cells of the cœlomic epithelium covering the face of the septum, which are much increased in size and richly ciliated (*lp. gst. d.*). These ciliated cells cover a large part of the anterior face of the posterior septum. The lower portion of the genital funnel (*lp. gst. v.*) is composed of ciliated cells attached to the lower lip of the nephridiostome, which form a membrane hanging freely in the cavity of the segment, and with the upper lip constituting a funnel-shaped structure surrounding the nephridiostome. This genital funnel (gonostome) is composed of cells of quite different structure to those of the nephridium, and the line of demarcation between the nephridiostome and gonostome

is well marked. Fig. 56 shows the appearance of these cells. Their protoplasm is clear, staining only feebly, and contains no granules such as are found in the nephridial epithelium. The cilia are long and their bases extend into the cell-body as deeply staining rods.

The funnels as well as the nephridiostomes and upper portions of the nephridial tubes are often filled with the genital products.

From the description above given it will be seen that in these genital segments we have to do with a compound organ, consisting of a nephridium and a genital funnel combined, which Goodrich, to whose very valuable papers we are indebted for much of our recent knowledge of similar structures amongst polychætes, has termed nephromixia.

Genital Products.

The genital products in Pœcilochætus are first found in the seventeenth segment, and occur in every segment behind that, with the exception of the segments at the extreme end of the body.

Ova.—The gonads lie along the inner and upper sides of the nephridial tubes (Pl. 12, figs. 58 (horizontal), 59 (transverse) and 60). As the ova increase in size they separate off from the gonads and pass upwards into the general body-cavity, where the process of maturation continues (fig. 59).

In their earliest recognisable stages (fig. 60) the developing ova appear simply as a number of enlarged nuclei, lying in a mass of cell substance in which no definite cell outlines are shown in the preparations. As the nucleus and cell-body enlarge, the individual ova become clearly marked out by a definite cell membrane, although for a time they continue to adhere together. The nucleus develops one large, deeply staining nucleolus and a number of smaller granules of chromatin (fig. 61). A well-developed yolk nucleus, horse-shoe shaped in section, forms a cap over about one half of the nucleus. This yolk nucleus consists of deeply staining granules (fig. 60 and 61, *yk. n.*) which in methyl-blue-eosin

preparations stain blue, in contrast to the nucleolus and chromatin granules, which stain red. It disappears as the egg continues to mature, when the whole of the protoplasmic contents of the egg becomes crowded with yolk granules, which stain blue in the preparations (figs. 62, 63 and 64).

The ripe eggs are lenticular in shape, the long diameter being about double the short. Around the line of greatest circumference there is a single row of vesicles, seen clearly in the optical section of a fresh egg represented in fig. 64. These vesicles are pear-shaped and open on the exterior surface of the egg by means of fine tubes passing through the thick egg membrane. In fresh eggs the vesicles look more clear and transparent than the general egg substance; in sections a small, shrunken mass of slightly staining substance appears in the centre of each (figs. 62 and 63). The function of these vesicles is unknown, though their appearance suggests that they may contain a fluid which is at some stage secreted on to the surface of the egg. That the vesicles are intimately connected with the egg membrane is shown by the fact that when the protoplasmic contents of the egg shrink, as they do when the egg is allowed to remain soaking for some time in sea water, the vesicles completely retain their position around the circumference of the egg membrane, their bases being connected by threads of protoplasm with the shrunken mass of the cell contents. This is shown in fig. 65, drawn from a fresh egg which had remained for some hours in sea-water.

Vesicles similar to those just referred to were described and figured by Claparède (1868) in Nerine cirratulus, in which form one circle of them is found round the equator, just as in the eggs of Pœcilochætus. In Nerine auriseta, on the other hand, Claparède found three irregular rows of similar vesicles arranged round the greatest circumference of the elliptical eggs. In neither case, however, does Claparède describe the fine tubes which place the vesicles in communication with the exterior. The following observation, which he records concerning the eggs of Nerine auriseta, is of interest:—"L'action d'une faibles solution de carminate

d'ammoniaque les modifie d'une manière remarquable. Elles se colorent assez rapidement en rouge intense, tandis que le vitellus ne se teint qu'en rouge pâle, et que le chorion reste parfaitement incolore." This observation appears to me to agree better with the suggestion made above that the vesicles may contain a secretory product than with the view set forth by Claparède:—"Je ne puis m'empêcher de supposer que ces vésicules (ou peut-être mieux ces sphères protoplasmiques) jouent un rôle important dans la formation du blastoderme" (Claparède, 1868, p. 333).

The egg membrane of the ripe egg of Pœcilochætus is very thick and stains deeply (blue in methyl-blue-eosin preparations). Its surface is ornamented by raised lines, which form an irregular pattern upon it (fig. 66, from a fresh egg). These lines or ridges are clearly visible in sections (figs. 62 and 63).

The germinal vesicle is large, its diameter being little less than the smaller diameter of the egg. It contains one large nucleolus, which is composed of a larger and a smaller spherical portion (cf. fig. 64, from a fresh egg, and fig. 63, from a section). Fig. 62 shows a condition of the nucleolus which is very often seen in preserved material. It here consists of a very deeply staining portion, which takes the form of a cap resting upon a more or less spherical, transparent vacuole. Such a form of the nucleolus is not uncommon in the eggs of other animals (for literature see Korschelt and Heider, 1902). When the nucleolus is in the state just described, a number of other deeply staining granules are present in the germinal vesicle.

Nothing has been ascertained as to the history of the eggs after they leave the body of the worm.

The Spermatozoa.—The place of origin of the male germinal cells is less restricted than that of the female. They sometimes arise, like the ova, from the cœlomic epithelium which surrounds the nephridial tube, but may also be derived from cœlomic epithelium in other parts of the segment, more especially from that of the anterior septum. In ripe males the body-cavity in the genital segments is filled

with a mass of sperm-cells in various stages of development and of spermatozoa.

The spermatozoa (fig. 53) have pear-shaped heads, rounded in front, and with straight posterior ends, to which the flagella are attached. A deeply staining portion at the posterior end of the head (*mp.*) probably represents the "middle-piece."

The Divisions of the Body.

Now that a description has been given both of the external characters and of the internal anatomy of Pœcilochætus, we are in a position to discuss more fully the question of the regions into which the body of the worm can properly be divided. These are (1) the prostomium, or head; (2) an anterior region, from the first segment to the eleventh; (3) an intermediate region, comprising segments 12, 13, 14, 15 and 16; (4) a genital region commencing at segment 17 and continuing backwards until it passes gradually into (5) the terminal region, or tail segments, and (6) the pygidium.

1. The prostomium, or head, has already been described. To it must be reckoned the median tentacle, the palps, the nuchal organ and two pairs of eyes.

2. The anterior region (segments 1 to 11) is characterised by the straight, muscular, cylindrical dorsal vessel; by the straight and muscular œsophagus and pharynx; by the presence, excepting in segments 1 and 2, of nephridia with nephridiostomes, but without genital funnels; by the absence of gonads; by the backward extension of the septa separating the body segments (segments 7 to 11), and consequent great elongation of the lateral blood-vessels which run in these septa; by the great development of the blind, finger-shaped vessels given off from each lateral vessel into the segment behind; by the peculiar modification of the parapodial cirri (segments 7 to 11); by the absence of hairy bristles; and by the pear-shaped lateral sense-organs protruding from the surface of the body.

It will be noted that the hindermost segments of this region (7 to 11) have several characters which distinguish them from those in front. These are the backward extension of the septa, the presence of spiny bristles, which are absent in segments 1 to 6 (excepting for one bristle in each parapodium of segment 2), and the peculiar modification of the parapodial cirri, which character in P. fulgoris and in the larvæ from Norway and Normandy is, according to Claparède, confined exclusively to these segments, as it is also in the pelagic larvæ of Pœcilochætus found at Plymouth.

3. The intermediate region (segments 12 to 16) is chiefly noteworthy from the presence of the large, contractile, lateral pouches of the dorsal vessel, which are found in its first four segments. The nephridia are still without genital funnels, and no gonads are developed. In the adult P. serpens the modified parapodial cirri of segments 7 to 11 extend back to segments 12 and 13; but this is not the case in P. fulgoris nor in any known larvæ of Pœcilochætus. In the latter the cirri of all the segments in this region have the conical form found in the genital region, and this is true also for the cirri of segments 14, 15 and 16 of P. serpens.

In segments 12, 13, 14 and 15 the segmental enlargements of the alimentary canal commence to appear, becoming more pronounced in each succeeding segment, whilst in segment 16 these enlargements are fully developed and the intestine is completely surrounded by a blood-sinus, the dorsal vessel ceasing to exist.

The hairy bristles of the genital segments are absent in this region (12—16), and the lateral organs still protrude from the body surface as in the anterior region.

4. The genital region, from segment 17 to within about thirty segments of the end of the body of a full-grown worm, is characterised by the presence of gonads and well-developed genital funnels; by the large intestinal pouches and intestinal blood-sinus; by the presence of large numbers of well-developed hairy bristles (figs. 14 and 16) and of flattened, membranous, spined bristles (fig. 17), which commence sud-

denly in segment 17; by the comparatively small size and conical shape of the parapodial cirri; by the change in the character of the lateral sense-organs, which no longer protrude beyond the body-wall; and by the presence (commencing on the twenty-first segment) of gill filaments on the posterior faces of the parapodia.

5. The terminal region, or tail segments, may be said to commence about the thirtieth from the end of the body, though the line of demarcation is not very definite. The segments are at first characterised by the presence of stout bristles with brush-like ends (fig. 18), instead of large hairy bristles; by the change in the character of the lateral organs; and in the last sixteen or seventeen segments, by the modification of the notopodial bristles into large curved hooks lying on the dorsal surface of the body (fig. 6).

6. The pygidium is characterised by the lobes surrounding the anus and by the two pairs of anal cirri.

Parasites.

In the body-cavity of almost every adult specimen of Pœcilochætus examined there occurred one or more examples of a parasitic Trematode. These were always encysted, and were readily recognised by the two large suckers.

Systematic Position.

The Family **Disomidæ**, Mesnil.

Mesnil (1897) formed the family Disomidæ for the reception of the two genera Disoma and Pœcilochætus.

The genus Disoma was founded by Oersted (1844), who described and figured one species, Disoma multisetosum. This species was again found by Möbius (1873), who gives further details of its anatomy and some figures.

Michaelsen (1897) was the first to obtain complete specimens of the worm, and he shows that the tail end of a specimen described and figured by Levinsen (1883) under

the name Trochochæta Sarsi almost certainly belongs to Oersted's species, Disoma multisetosum.

Mesnil (1897), from an examination of the type specimens, confirms the specific identity of Michaelsen's specimens with those of Oersted. He gives some further details, with figures, of the structure of the worm, and expresses the opinion that Thaumastoma singulare, described by Webster and Benedict (1884, p. 737), from the American coast, is the same species.

Claparède (1868, p. 337) discussed the relations of Disoma with Polydora and with Chætopterus, being inclined to place it near to the latter.

Levinsen (1883, p. 106) pointed out that the two genera, Disoma and Pœcilochætus, were closely related to each other, and emphasised their resemblance to the Spionidæ.

Adopting Levinsen's view of the relation of the two forms, "sans avoir pourtant une conviction bien ferme," Mesnil (1897) placed both in his family Disomidæ, which he characterises as follows:

Prostomium very simple, with two long tentacular palps analogous to those of the Spionids. Parapodia biramous, at any rate in the anterior region, always with simple bristles. Bristles of various kinds, especially large spiny bristles, hairy bristles, and large lancet-shaped bristles. Stout hooks (soies aciculaire) in the neuropodia of segments 2, 3, and even 4. Never two regions of the body clearly marked off. Ventral and dorsal cirri elongate or fimbriate.

Mesnil considers this family as intermediate between the Spionidæ and Chætopteridæ, being somewhat nearer to the latter. He also points out that in certain characters the two genera show some affinities with the Aphroditidæ and Amphinomidæ, and that this is particularly true of Pœcilochætus on account of the median tentacle and the large spiny bristles. The two long palps and the tendency of the first segment to enclose the prostomium also point in the same direction.

My own observations on Pœcilochætus and a study of the different descriptions of Disoma lead me to agree with

Levinsen and Mesnil in regarding the two genera as nearly related, and Mesnil's foundation of the family Disomidæ appears justified.

I am inclined, however, to consider this family as more closely allied to the Spionidæ than to any other Polychæte family, as was maintained by Claparède and Levinsen. In addition to the presence of the large palps this view is supported by the line of vesicles surrounding the eggs, a striking character which appears to be found only amongst the Spionidæ. The median tentacle of Pœcilochætus in all probability represents the fusion of two lateral tentacles, and may be homologous to the two lateral processes at the front end of the head in such forms as Nerine (Scolelepis) vulgaris. The great development of the nuchal organ might be held to mark Pœcilochætus off from the Spionidæ and to bring it nearer to the Amphinomidæ, where, according to Racovitza, the caruncle is a very large nuchal organ. This argument, however, can have little weight, as the nuchal organ varies greatly in its development in closely allied forms within other families (e. g. Syllidæ, Phyllodocidæ), and the organ is present in the form of ciliated grooves at the posterior end of the head of Polydora, as I have been able to demonstrate on sections.

The Species of Pœcilochætus.

The chief points in which Claparède's description of P. fulgoris (Claparède in Ehlers, 1874) differs from the description given in the present paper of the Pœcilochætus found at Plymouth are as follows:

1. The large palps of the Plymouth species are not described in P. fulgoris. This, however, is not surprising, and is certainly due to the imperfection of the specimens, it being exceedingly difficult to prevent the worm from throwing these palps off.

2. The nuchal organ, though indicated by Claparède both in his figure and text, appears much less developed in

P. fulgoris. Here, again, imperfect preservation may account for the difference.

3. The tubercles (openings of epithelial glands), which cover both the dorsal and ventral surfaces of Pœcilochætus fulgoris, are scarcely represented on the dorsal surface of Plymouth specimens, though moderately common on the ventral.

4. Only one pair (dorsal or posterior) of eyes is described by Claparède, the ventral (anterior) pair not having been observed.

5. Claparède describes the buccal segment as having a single cirrus on each side. The rudimentary dorsal cirrus was either not present or was overlooked.

6. The long dorsal cirrus of segment 5 is not described or figured by Claparède. The cirri of the seventh to the eleventh segments differ in shape from the others, being flask shaped with long, stiff necks in Claparède's specimen, whilst in the Plymouth specimens this character is constant for segments from the seventh to the thirteenth. As, however, all larvæ seen at Plymouth agree with P. fulgoris in this respect, the difference may be due to the fact that Claparède's specimens were not adult.

7. The second, the third, and the fourth segments of the "Lightning" specimens have short, stout spines in the neuropodium; in the Plymouth specimens such spines are confined to the second and third segments.

The differences expressed under the headings (3), (6) and (7) appear to render it necessary to regard the Plymouth specimens, at least provisionally, as belonging to a new species for which I propose the name Pœcilochætus serpens.

Definitions.

Family **Disomidæ**, Mesnil.

Polychæta having a simple prostomium without tentacles or with one median tentacle, and with four simple eyes. A pair

of large palps capable of great elongation. Mouth ventral with a short proboscis. Parapodia of the first segment greatly developed and directed forwards, provided with long chætæ which meet in front of the head. Parapodia with well-developed dorsal and ventral cirri. Chætæ simple, and either smooth or bearing hairs or spines. Neuropodia of the second and third (and even fourth) segments, with three or four short, stout bristles or hooks. Notopodial chætæ of the terminal segments modified into stout, strong hooks or spikes situated on the dorsal surface of the body. Distinct posterior (genital) region of the body commencing at the seventeenth segment. Segments from about the twentieth backwards having three or four filamentous gills.

Genus **Disoma**, Oersted.

Polychæta having the general characters of the family Disomidæ. Prostomium without tentacles. Both neuropodial and notopodial cirri well developed in the first segment. Notopodial cirri from the third to the sixteenth segments having the form of elongated, crenated plates, running transversely on the dorsal surface. Notopodial and neuropodial cirri from segment 17 backwards conical. Gills on either side of the mid-ventral line commencing at the twentieth segment. Notopodial bristles of the most posterior segments stout spines arranged in star-like clusters on the dorsal surface of the body.

One species only known—

Disoma multisetosum, Oersted.

Synonyms: Trochochæta Sarsi, Levensen.

Thaumastoma singulare, Webster and Benedict.

Genus **Pœcilochætus**, Claparède.

Polychæta having the general characters of the family Disomidæ. Prostomium with one anterior median tentacle. Nuchal organ in the form of three lobes or tentacle-like

processes arising from the posterior end of the prostomium. Neuropodial cirrus of the first segment well-developed, notopodial cirrus rudimentary. Neuropodial and notopodial cirri from the seventh to the eleventh (or to the thirteenth) segments flask-shaped, with long, stiff necks. Gills on the parapodia from segment 21 backwards. Chætæ from the seventeenth segment backwards mostly with long hairs; those of the notopodium in the most posterior segments stout hooks, forming transverse rows on the dorsal surface of the body. Anus dorsal, with two long and two short cirri. Dorsal blood-vessel with large, lateral pouches in segments 12, 13, 14, and 15.

Two species (provisionally)—

Pœcilochætus fulgoris, Claparède. Anterior dorsal surface of the body richly provided with tubercles. Parapodial cirri of segments 7 to 11 different from those on the rest of the body, being flask-shaped, with long, stiff necks. Second, third and fourth segments with short, stout spines in the neuropodium. Nuchal organ moderately developed (?).

Pœcilochætus serpens, n. sp. Anterior dorsal surface smooth, with few tubercles. Parapodial cirri of segments 7 to 13 (in the adult) different from those on the rest of the body, being flask-shaped, with long, stiff necks. Second and third segments only with short, stout spines in the neuropodium. Nuchal organ greatly developed, forming three long tentacle-like processes.

Literature.

1843. Oersted.—'Ann. dan. conspectus. I. Maricolæ,' 1843, p. 41.

1844. Oersted.—"Zur Classification der Annulaten," 'Wiegm. Arch. f. Naturgesch,' p. 107.

1863. Claparède.—'Beobachtungen über Anatomie und Entwicklungsgeschichte wirbellosen Thiere an der Küste von Normandie angestellt,' Leipzig.

1868. Claparède, Ed.—"Les Annélides Chétopodes du Golfe de Naples," Genève.

1873. Möbius, K.—"Die Expedition zur Untersuchung der Ostsee," 'Comm. zur Untersuchung der deutschen Meere,' i, p. 108.

1874. Ehlers, E.—"Beiträge zur Kenntniss der Verticalverbreitung der Borstenwürmer im Meere," 'Zeitsch. wiss. Zoologie,' vol. xxv.

1879. Eisig, H.—"Die Seitenorgane und becherförmigen Organen der Capitelliden," 'Mitth. Zool. Stat. Neapel.,' Bd. i, p. 278.

1880. Engelmann, T. W.—"Zur Anatomie und Physiologie der Flimmerzellen," Pflüger's 'Archiv,' vol. xxiii.

1883. Levinsen, G. M. R.—'Systematisk-geografisk Oversigt over de Nordiske Annulata, Gephyrea, Chætognathi og Balanoglossi, Kjobenhavn.'

1886. Viguier.—"Étude sur les animaux inférieurs de la baie d'Alger. II. Recherches sur les Annélides pélagiques," 'Arch. Zool. Exp. et Gen.,' Ser. 2, vol. iv.

1887. Eisig, H.—"Monographie der Capitelliden des Golfes von Neapel," 'Fauna und Flora des Golfes von Neapel.'

1888. Heidenhain, R.—"Beiträge zur Histologie und Physiologie der Dünndarmschleimhaut," Pflüger's 'Archiv,' vol. xliii, Suppl. Heft.

1892. Greenwood, M.—"On Retractile Cilia in the Intestine of Lumbricus terrestris," 'Journal of Physiology,' vol. xiii.

1893. Malaquin, A.—"Recherches sur les Syllidiens," 'Mem. Soc. Sci. et Art. Lille.'

1894. M'Intosh, W. C.—"A Contribution to our Knowledge of the Annelida," 'Quart. Journ. Micr. Sci.,' vol. xxxvi.

1896. Benham, W. B.—"The Blood of Magelona," 'Quart. Journ. Micr. Sci.,' vol. xxxix.

1896. Gravier, C.—"Recherches sur les Phyllodociens," 'Bull. Sci. France et Belg.,' vol. xxix.

1896. Racovitza, E. G.—"Le Lobe Céphalique et l'Encéphale des Annélides Polychètes," 'Arch. Zool. Exp. et Gen.,' Ser. 3, vol. iv.

1897. Hesse, R.—"Untersuchungen über die Organe der Lichtempfindung bei niederen Thieren. II. Die Augen der Plathelminthen, insonderheit der tricladen Turbellarien," 'Zeitschr. wissensch. Zool.,' vol. lxii.

1897. Mesnil, F.—"Études de Morphologie externe chez les Annélides," 'Bull. Sci. France et Belg.,' vol. xxx.

1897. Michaelsen, W.—"Die Polychæten Fauna der deutschen Meere," 'Wissen. Meeresuntersuch' ii.

1899. Hesse, R.—"Untersuchungen über die Organe der Lichtempfindung bei niederen Thieren. V. Die Augen der polychäten Anneliden," 'Zeitschr. wissensch. Zool.,' vol. lxv.

1900. Goodrich, E. S.—"On the Nephridia of Polychæta," part iii, 'Quart. Journ. Micr. Sci.,' vol. xliii.

1901. Ashworth, J. H.—"The Anatomy of Scalibregma inflatum, Rathke," 'Quart. Journ. Micr. Sci.,' vol. xlv.

1902. Korschelt and Heider.—"Lehrbuch der vergleichenden Entwicklungsgeschichte der wirbellosen Thiere," 'Allgemeiner Theil.'

1902. Mann, G.—'Physiological Histology, Methods and Theory,' Oxford, Clarendon Press.

1903. Galvagni, E.—"Histologie des Genus Ctenodrilus, Clap.," 'Arbeit. Zool. Inst. Wien.,' vol. xv., part 47.

1903. Schepotieff, A.—"Untersuchungen über den feineren Bau der Borsten einiger Chætopoden und Brachiopoden," 'Zeitschr. wiss. Zool.,' vol. lxxiv.

1903. Launoy, L.—"Contribution à l'Étude des Phénomènes Nucléaires de la Sécrétion (Cellules à venin, Cellules à enzyme)," 'Ann. Sci. Nat. Zool.,' vol. xviii.

1904.—Brasil, L.—"Contribution a la Conaissance de l'Appareil digestif des Annélides Polychètes. L'épithelium intestinal de la Pectinaire," 'Archiv. Zool. Expér. et Gen.,' ser. 4, t. ii, 1904, p. 91.

EXPLANATION OF PLATES 7—12,

Illustrating Dr. E. J. Allen's paper on "The Anatomy of Pœcilochætus, Claparède."

List of Reference Letters.

b. lat. v. Branch of lateral blood-vessel passing into the next following segment. *br.* Brain. *c.* Cilia. *cal.* Callosities on cuticle. *com.* Œsophageal commissure. *cu.* Cuticle. *cu.g.* Thickened cuticular layer of nuchal groove. *dors. v.* Dorsal blood-vessel. *ep.* Epithelium. *ep. r.* Epithelial rim of lateral organ. *f.p.* Finger-shaped processes into which the backwardly directed branch of the lateral vessel breaks up. *g.* Masses of protoplasm in which granules are formed in the lip of the nephridiostome. *gang. plp.* Palp-ganglion. *g. l. o.* Ganglion-cells of lateral sense-organ. *g.f.* Giant-fibres. *gl.* Gland-cell. *gl. 1, gl. 2, gl. 3.* Three types of goblet-

shaped gland-cells of the œsophagus. *gst.* Gonostome. *i. bl. s.* Intestinal blood-sinus. *intest.* Intestine. *int. v.* Blood-vessel from walls of alimentary canal to ventral vessel. *lat. v.* Lateral blood-vessel. *l. o.* Lateral sense-organ. *lp. gst. d.* Dorsal lip of gonostome. *lp. gst. v.* Ventral lip of gonostome. *lp. nst.* Lip of nephridiostome. *l. r.* Long rods of lateral sense-organ, nuchal organ, and epithelium of œsophagus. *m. b.* Mid-brain. *m. f.* Muscle-fibres. *m. p.* Middle-piece of spermatozoon. *m. tent.* Median tentacle. *mth.* Mouth. *musc.* Muscle. *n.* Nucleus; *n.'* see page 97. *nh.* Supposed nuclei of hair-bearing cells. *n. p. 1, n. p. 2.* First and second roots of nerve of palp-ganglion. *nph.* Nephridial tube. *nr. c.* Neuropodial cirrus. *nst.* Nephridiostome. *nt. c.* Notopodial cirrus. *nuch.* Nuchal organ. *nuc. gan.* Nuchal ganglion. *œs.* Œsophagus. *p.* Process from callosity, see page 97. *p. bv.* Palp blood-vessel. *p. dv.* Lateral pouch of dorsal blood-vessel. *ph.* Pharynx. *plp.* Palp. *plp. div.* Diverticle of palp. *plp. v.* Palp-valve. *sep.* Septum dividing body segments. *sh.* Sensory hairs of lateral sense-organs. *sr.* Short rods of lateral organs, nuchal organ, and epithelium of œsophagus. *sr. 2.* Secondary layer of short rods in lateral organs. *v.* Point where pharynx joins intestine. *vent. v.* Ventral blood-vessel. *vl. seg. 14.* Valve in dorsal blood-vessel between segments 14 and 15 (open). *vl. seg. 15.* Valve in dorsal blood-vessel between segments 15 and 16 (closed). *yk. n.* Yolk nucleus.

All sections and the majority of the other figures were drawn with the camera lucida.

PLATE 7.

FIG. 1.—Anterior segments of Pœcilochætus serpens. The vascular system is somewhat diagrammatic, having been reconstructed from sections. × *ca* 25.

FIG. 2.—Parapodium 3, left side. × 66.

FIG. 3.—Parapodium 5, left side. × 66.

FIG. 4.—Parapodium 7, left side. × 66.

FIG. 5.—Parapodium 13, left side. × 66.

PLATE 8.

FIG. 6.—Terminal segments, dorsal view. × *ca* 50.

FIG. 7.—Head end, dorsal view. × *ca* 50.

FIG. 8.—Head end, ventral view. × *ca* 50.

PLATE 9.

FIG. 9.—Parapodium 14, left side. × 66.

FIG. 10.—Parapodium 18, left side. × 66.

Fig. 11.—Parapodium 30, left side. × 66.

Fig. 12.—Pœcilochætus serpens in burrow constructed in sand between two glass plates. Natural size.

Fig. 13.—Smooth bristle from parapodium 7. × 380.

Fig. 14.—Large, stiff, hairy bristle from parapodium 30. × 380.

Fig. 15.—Spined bristle from parapodium 10. × 380.

Fig. 16.—Small, flexible, hairy bristle from parapodium 30. × 380.

Fig. 17.—Membranous spined bristle from parapodium 20. × 380.

Fig. 18.—Bristle with hairy terminal brush from twentieth parapodium from end. × 380.

Fig. 19.—Stout hooks of the notopodium from the dorsal surface of the seventh segment from the end. × 100.

Fig. 20.—Transverse section of epithelium from the anterior dorsal surface. × 1180.

Fig. 21.—Transverse section of epithelium from the ventral surface, just behind the mouth. × 1180.

PLATE 10.

Fig. 22.—Unicellular epithelial gland from anterior portion of body. × 1180.

Fig. 23.—Section of gland-cells and tubercle from posterior end of body. × 1180.

Fig. 24.—Section of parapodial cirrus (about segment 20). × 220.

Fig. 25.—Cirrus from near tail of living worm.

Figs. 26—29.—Gland-cells from parapodial cirri. × 1180.

Fig. 30.—Transverse section through the base of the palps and palp-ganglia. × 135.

Fig. 31.—Transverse section of a gill filament. × 400.

Fig. 32.—Transverse section of the ventral nerve-cord. × 220.

Fig. 33.—Sagittal section through a ventral eye. × 640.

Fig. 34.—Horizontal section through a lateral sense-organ of the genital region. × 600.

Fig. 35.—Transverse section through a lateral sense-organ of the genital region passing through the anterior row of muscle-bands. × 690.

Fig. 36.—Transverse section through a lateral sense-organ of the genital region passing through the hair-bearing cells. × 690.

Fig. 37.—Transverse section through a lateral sense-organ of the genital region passing through the posterior row of muscle-bands. × 690.

FIG. 38.—Section through the extremity of a lateral sense-organ of the genital region in the longitudinal vertical (sagittal) plane of the body. × 375.

FIG. 39.—Section through a lateral sense-organ of the anterior region. × 690.

PLATE 11.

FIG. 40.—Section through a ciliated groove of the nuchal organ. × 640.

FIG. 41.—Enlarged view of ciliated cells of the nuchal organ. × 1180.

FIG. 42.—Sagittal section through the mouth and median tentacle. × 66.

FIG. 43.—Horizontal section through segments 8 to 14. × 42.

FIG. 44.—Transverse section through ciliated epithelium of the œsophagus. × 690.

FIG. 45.—Transverse section through epithelium of the intestine, when the latter is filled with food and digestion is actively going on. × 690.

FIG. 46.—Transverse section through epithelium of the intestine, when digestion is not active. × 690.

FIG. 47.—Sagittal section through the first sixteen segments. × 42.

FIG. 48.—Transverse section through anterior region, showing junction of the lateral vessels with the ventral vessel. × 42.

FIG. 49.—Transverse section through anterior region, showing cluster of finger-shaped processes uniting to the branch of the lateral blood-vessel. × 42.

FIG. 50.—Sagittal section through the dorsal blood-vessel in segments 14 and 15, showing the valves. × 132.

PLATE 12.

FIG. 51.—Sagittal section through two segments of the anterior region, showing the branch of the lateral vessel going into the segment behind and breaking up into finger-shaped processes. × 88.

FIG. 52.—Sagittal section of a nephridium and genital funnel of a male genital segment. × 212.

FIG. 53.—Spermatozoa. × 1770.

FIG. 54.—Sagittal section through the nephridiostome of an anterior segment. × 424.

FIG. 55.—Transverse section through the lip of the nephridiostome of an anterior segment. × 424.

FIG. 56.—Ciliated epithelium of the dorsal lip of a genital funnel × 1180.

FIG. 57.—Enlarged portion of the lip of a nephridiostome. The dark black dots stain red, the grey shading blue in methyl-blue-eosin preparations. × 1180.

FIG. 58.—Horizontal section through three genital segments, showing the relation of the ovary to the nephridium. × 66.

FIG. 59.—Transverse section through a genital segment. × 66.

FIG. 60.—Section through a nephridial tube, showing the development of the ova. × 380.

FIGS. 61—63.—Sections of ova in different stages of maturation. × 380.

FIG. 64.—Optical section of living mature ovum. × 212.

FIG. 65.—Optical section of living mature ovum after remaining some hours in sea-water. × 212.

FIG. 66.—Surface view of living mature ovum. × 212.

Fig 2

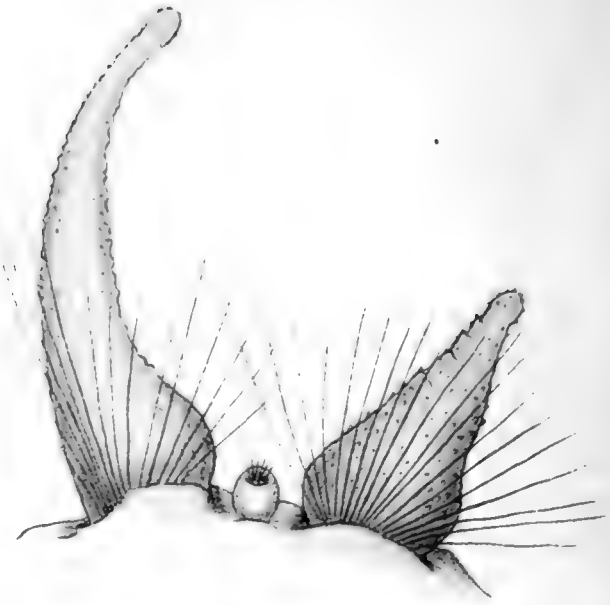
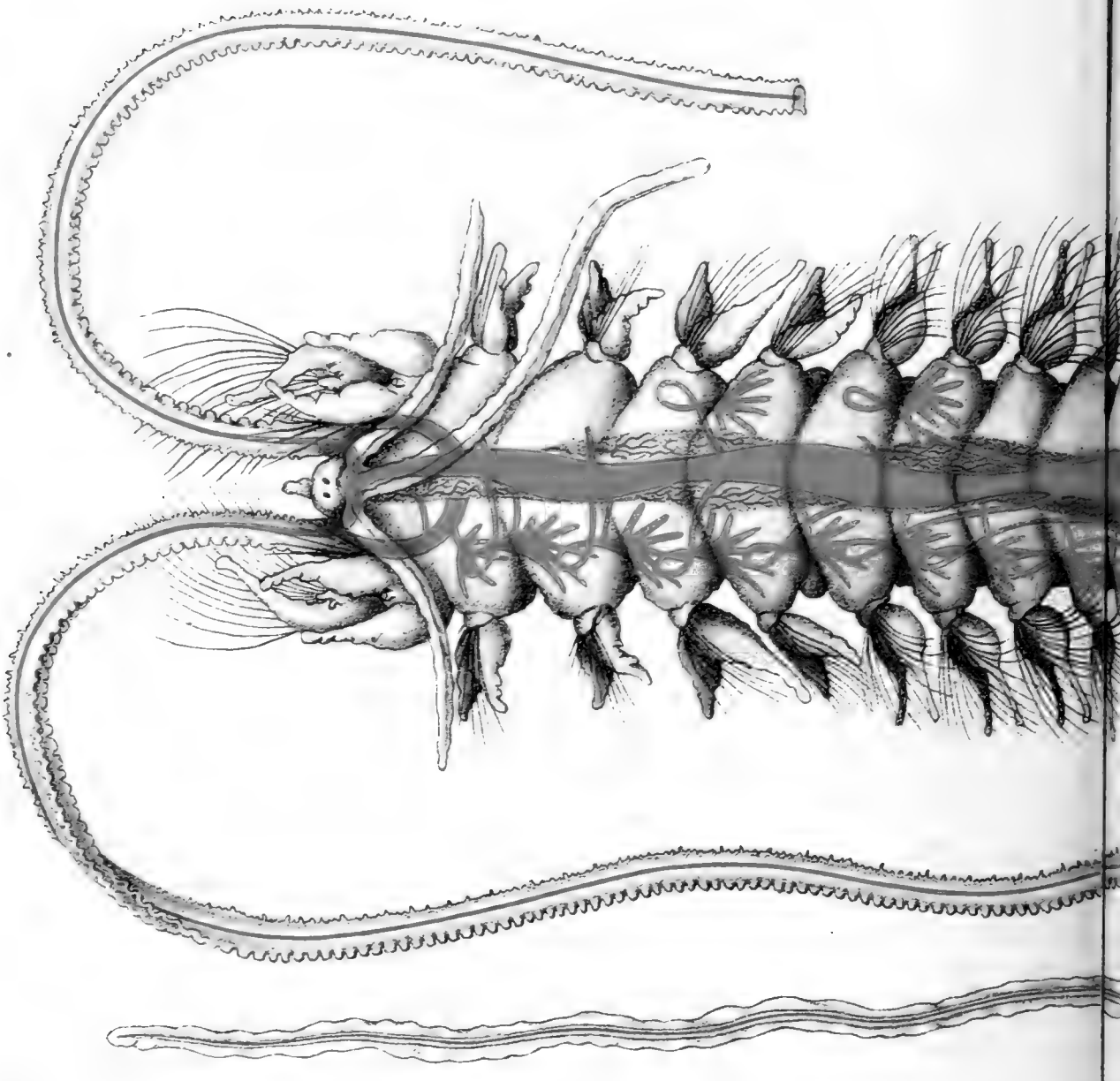
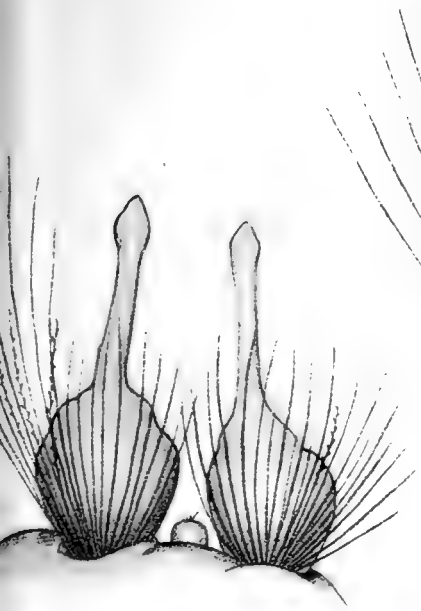


Fig 3





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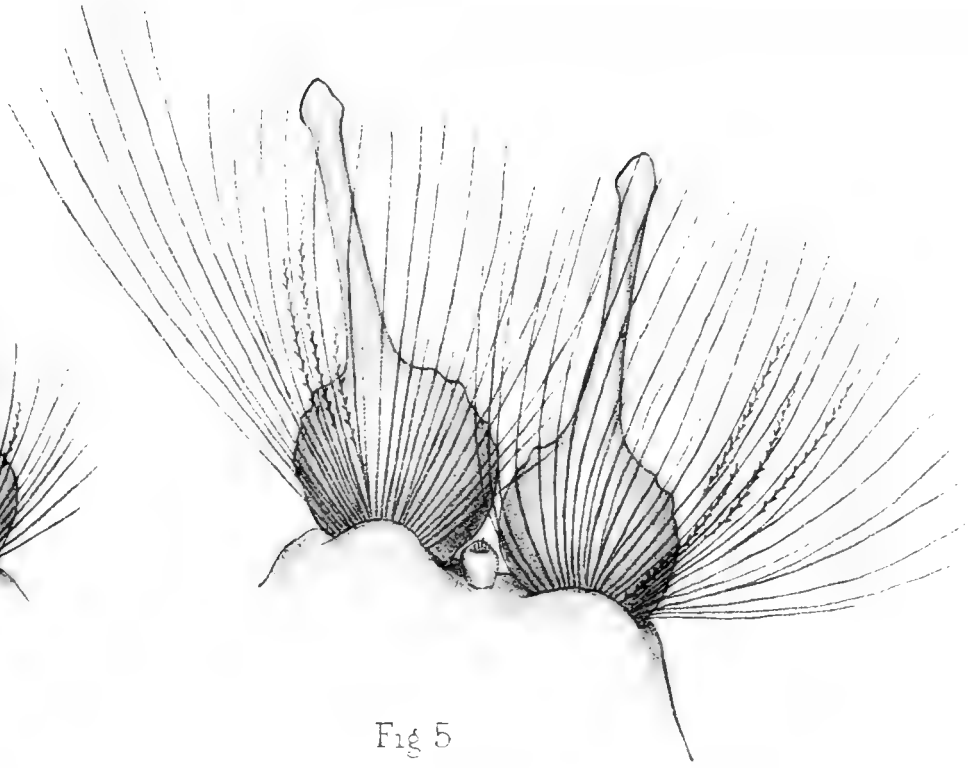
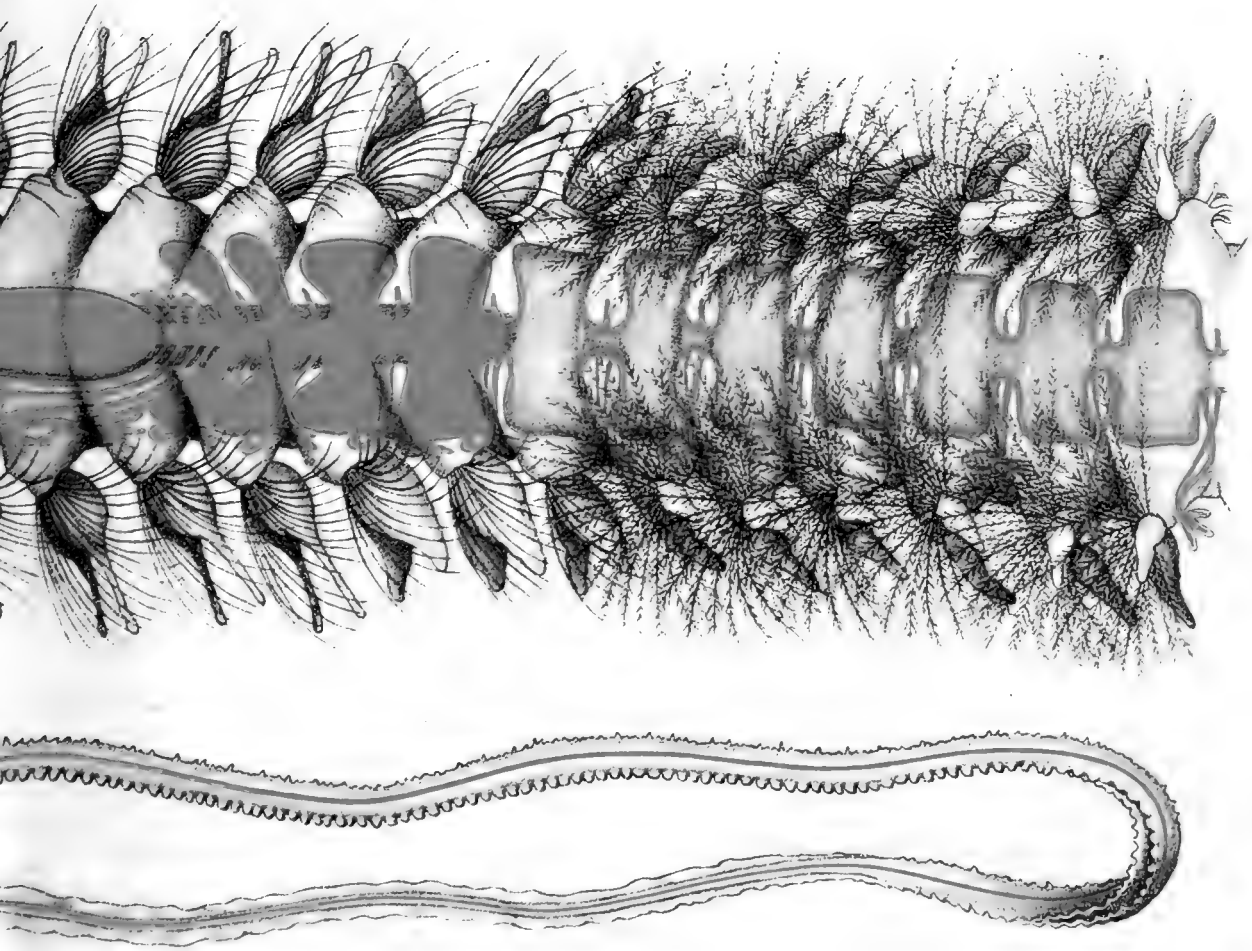


Fig 5



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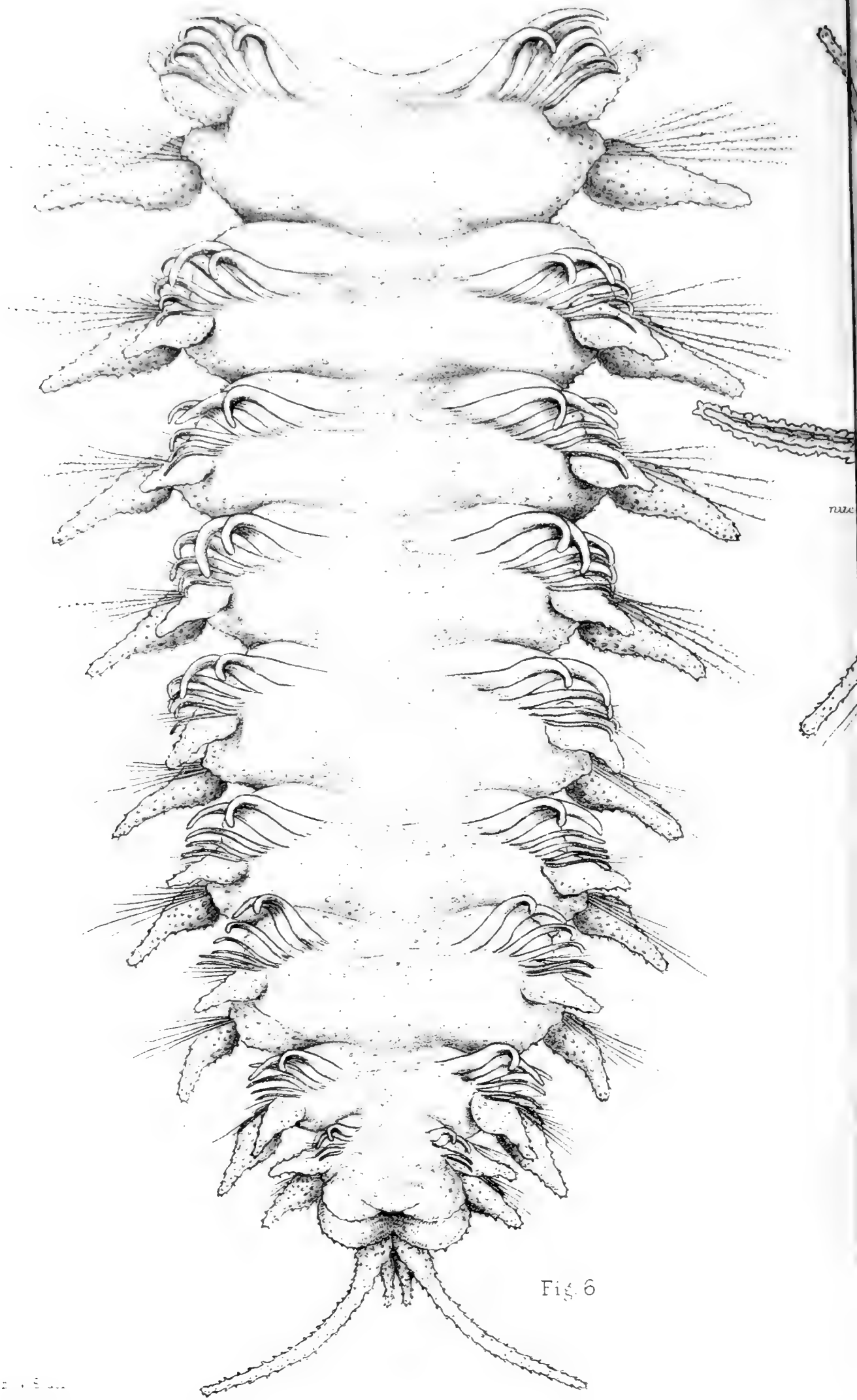


Fig. 6

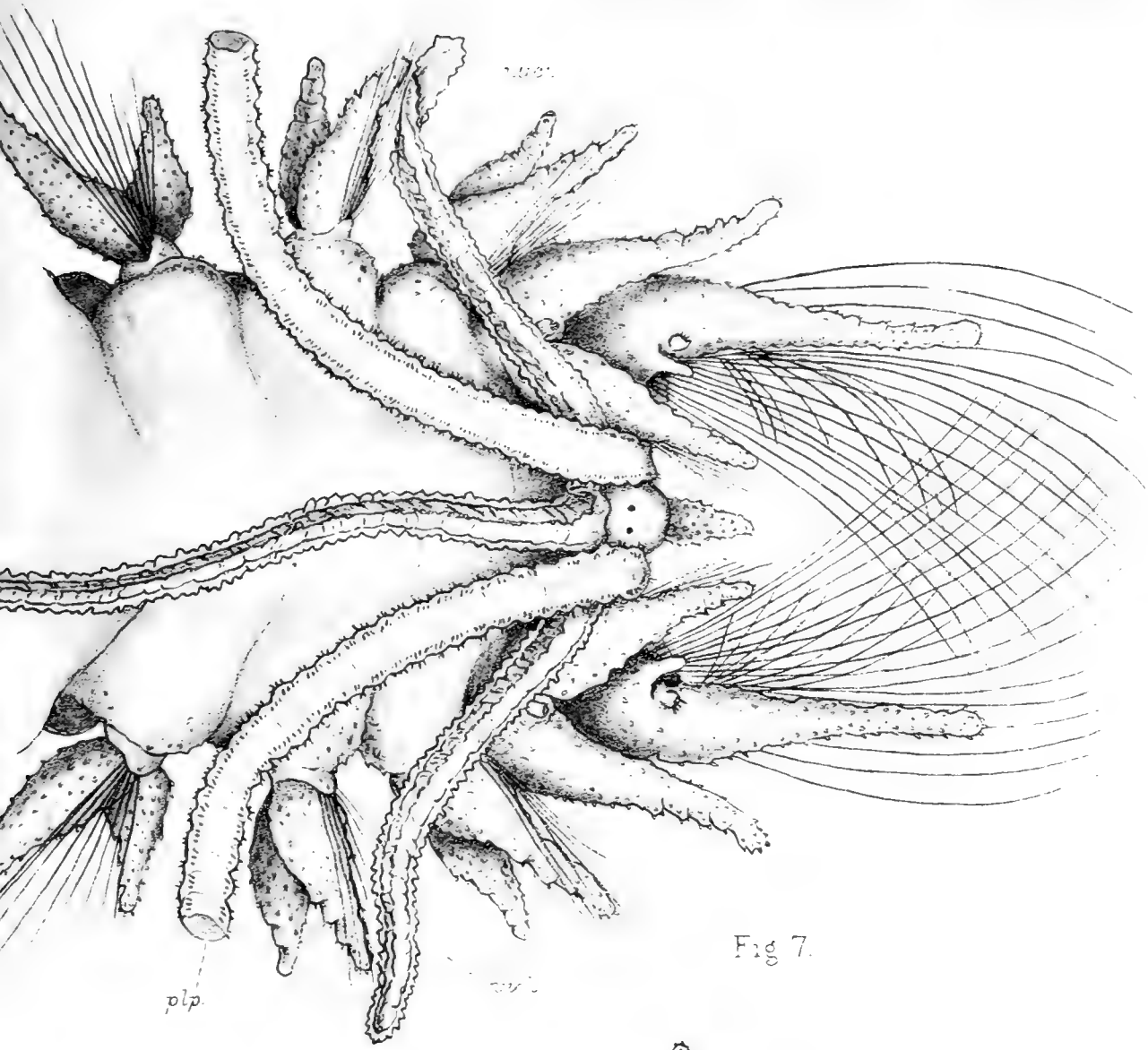
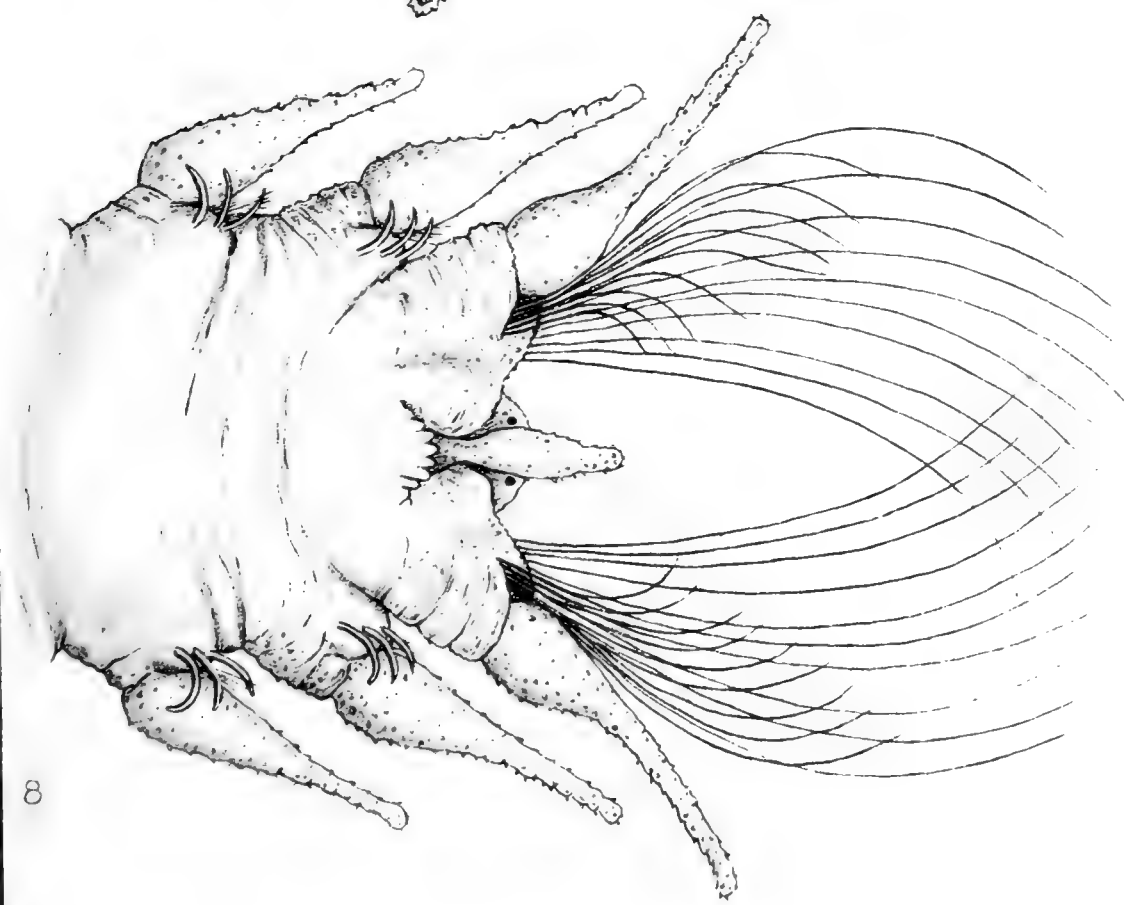


Fig 7.



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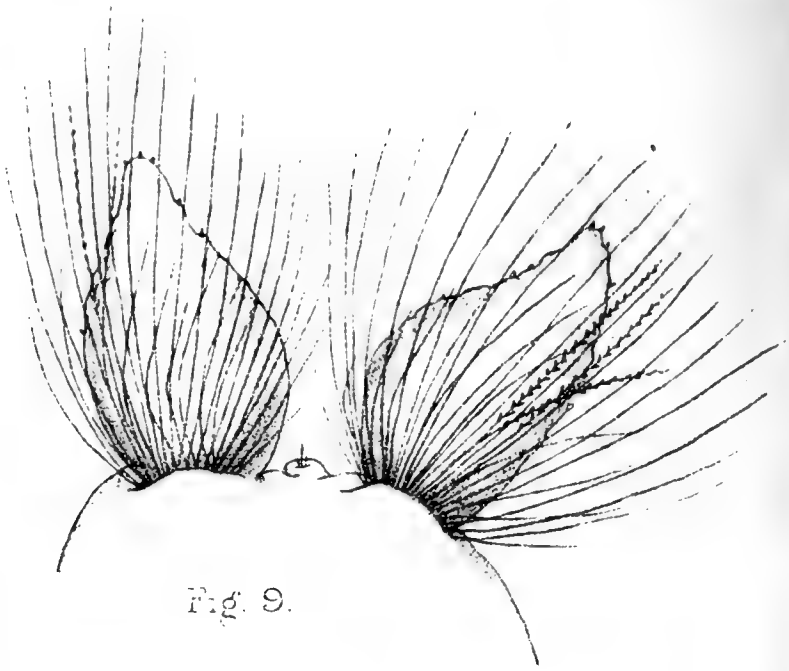

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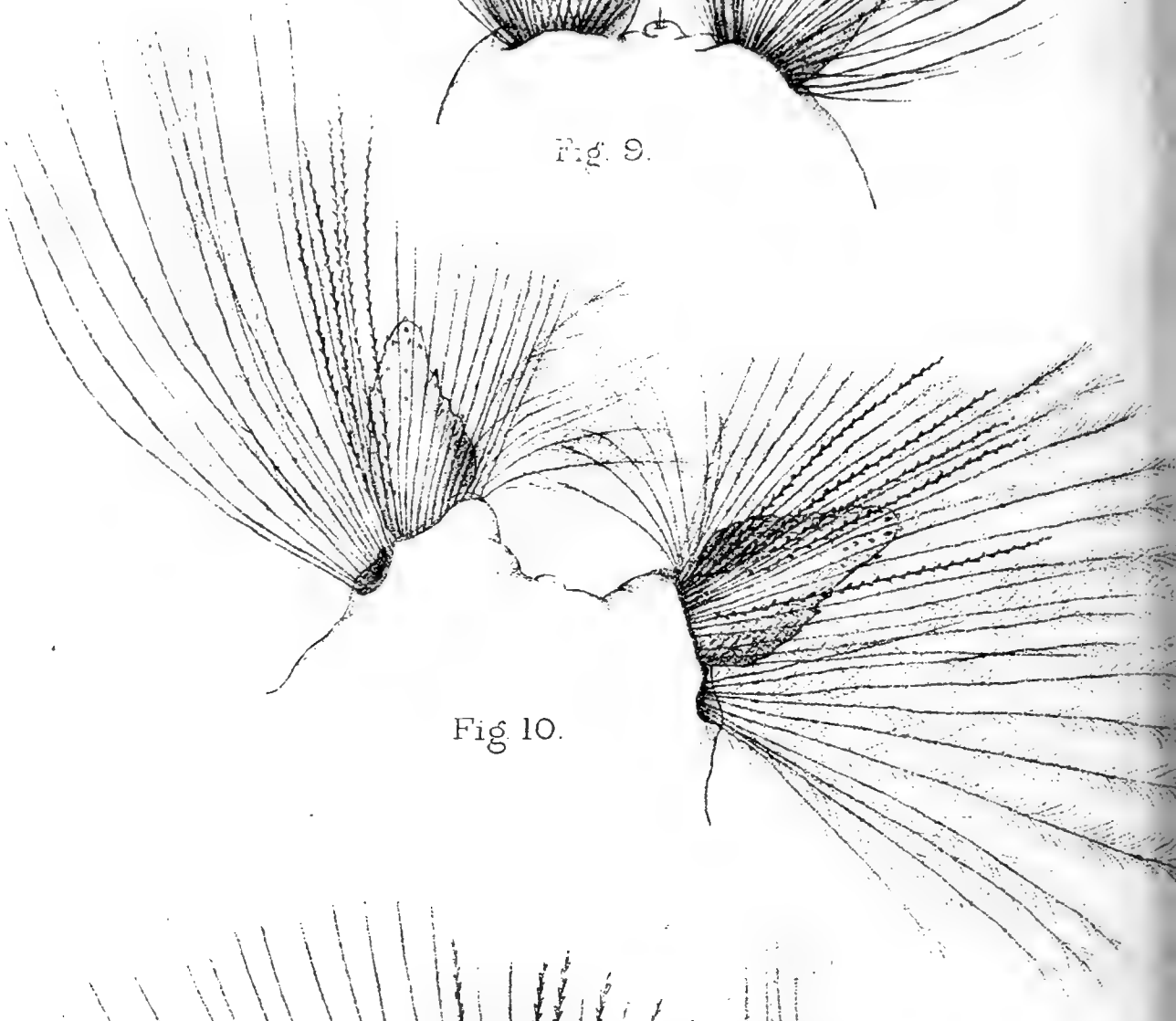

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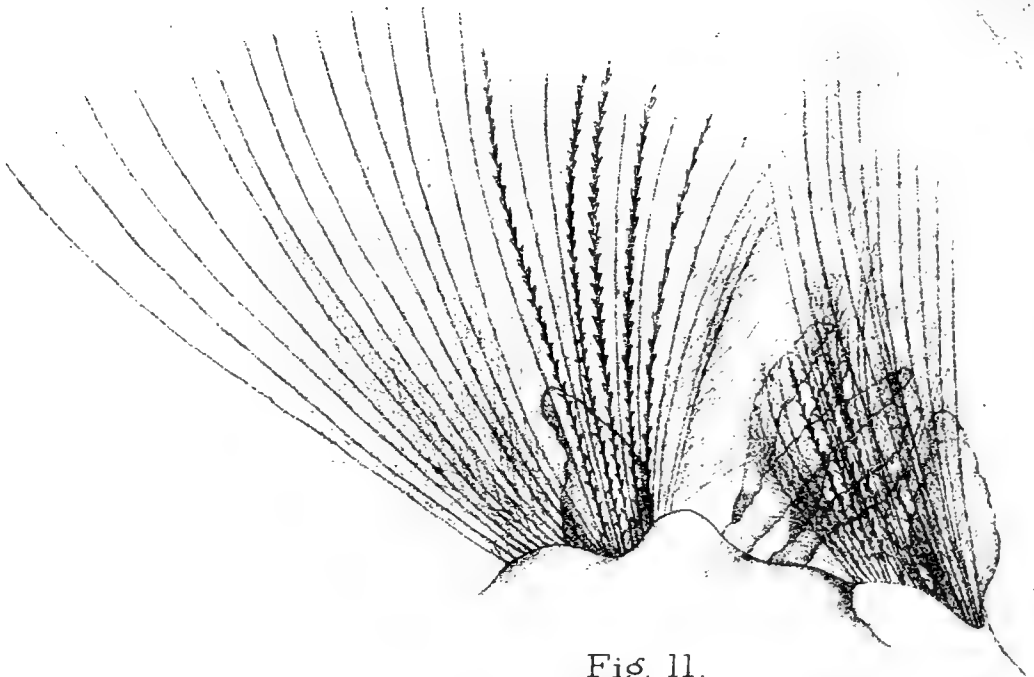

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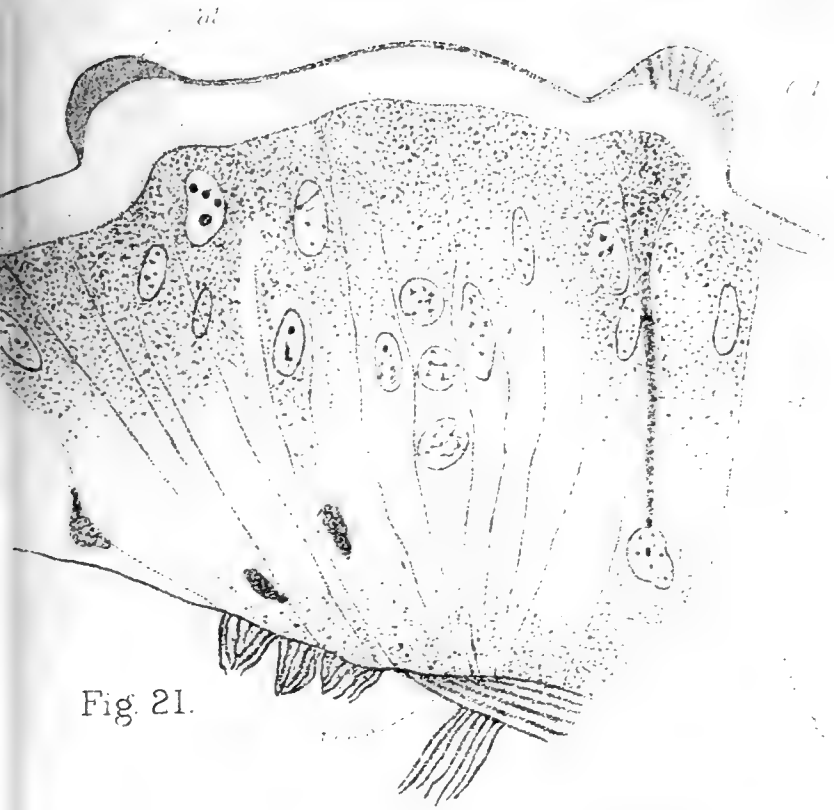

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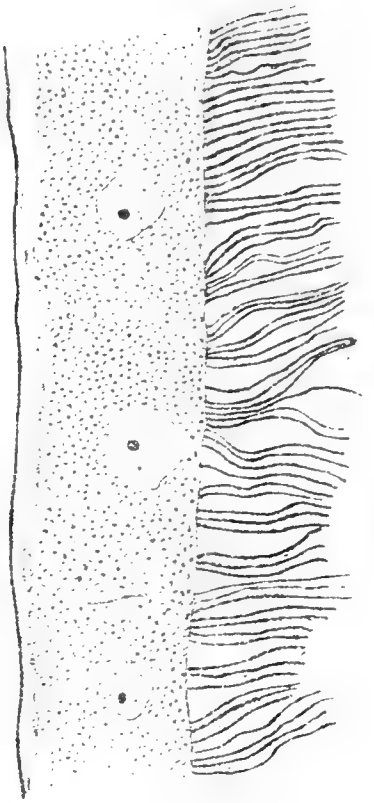
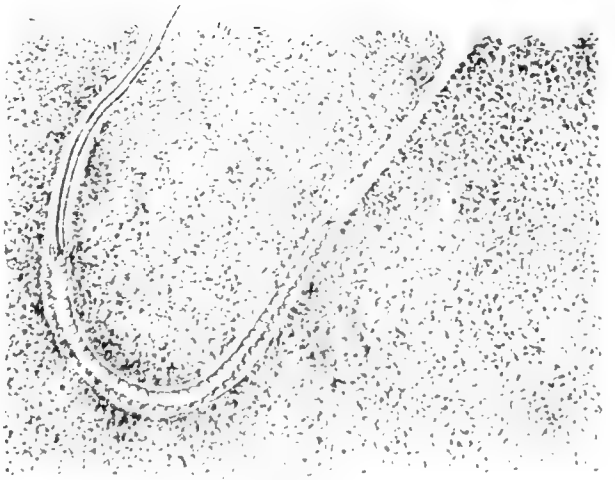


Fig. 20

Fig. 16

Fig. 18.

Fig. 19.



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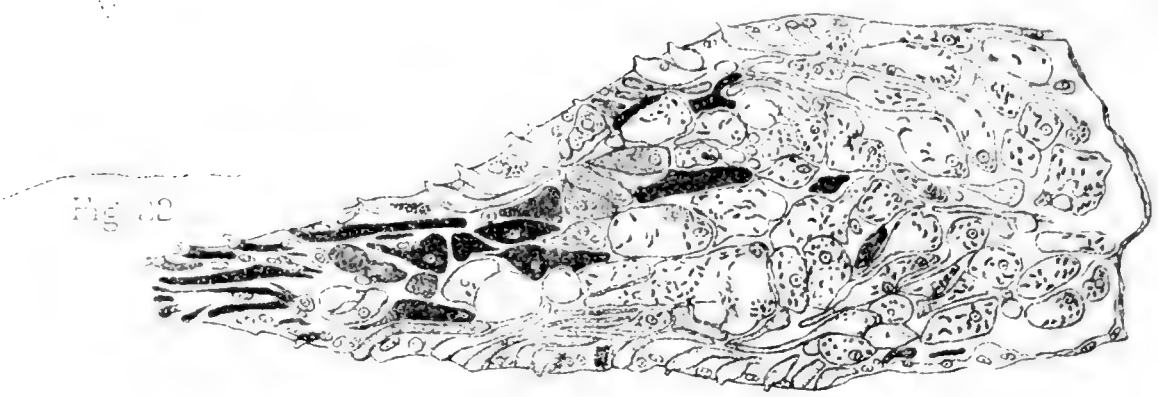

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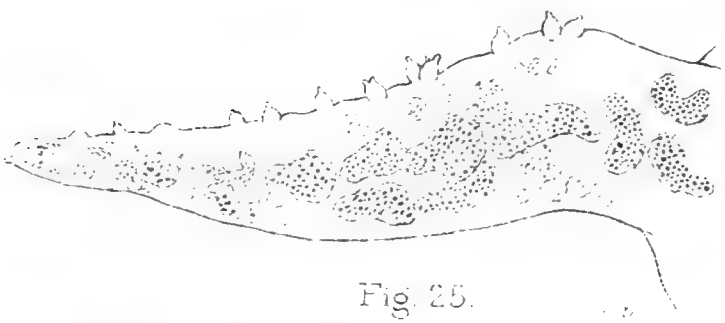

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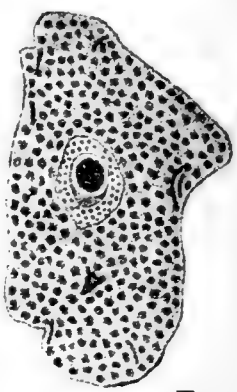

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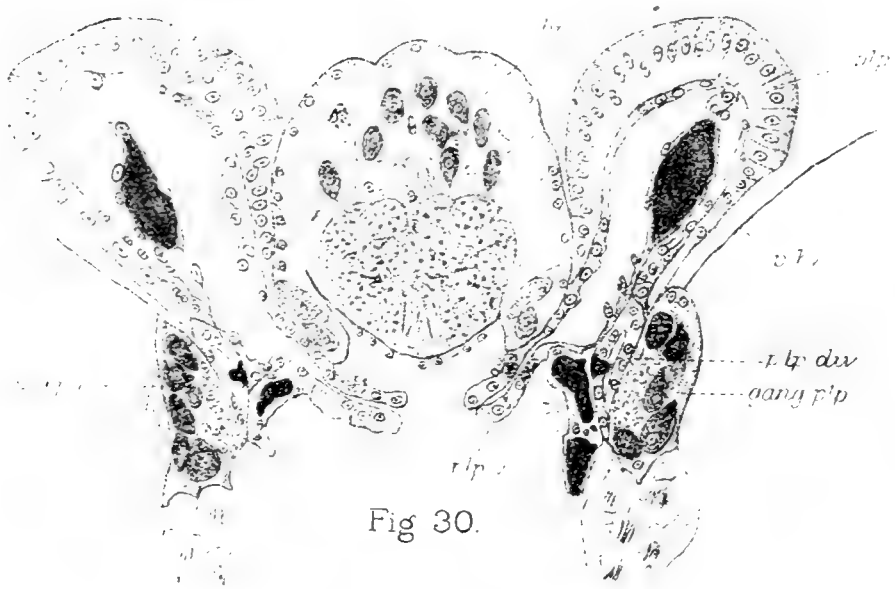

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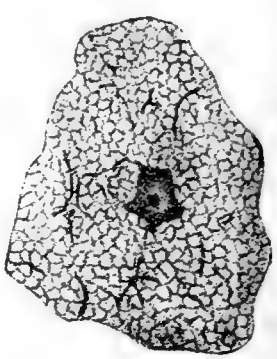

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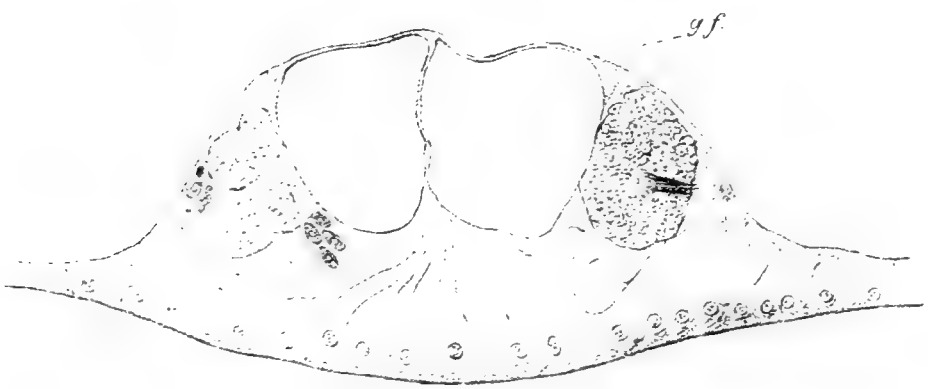


Fig. 32.

Fig. 33.

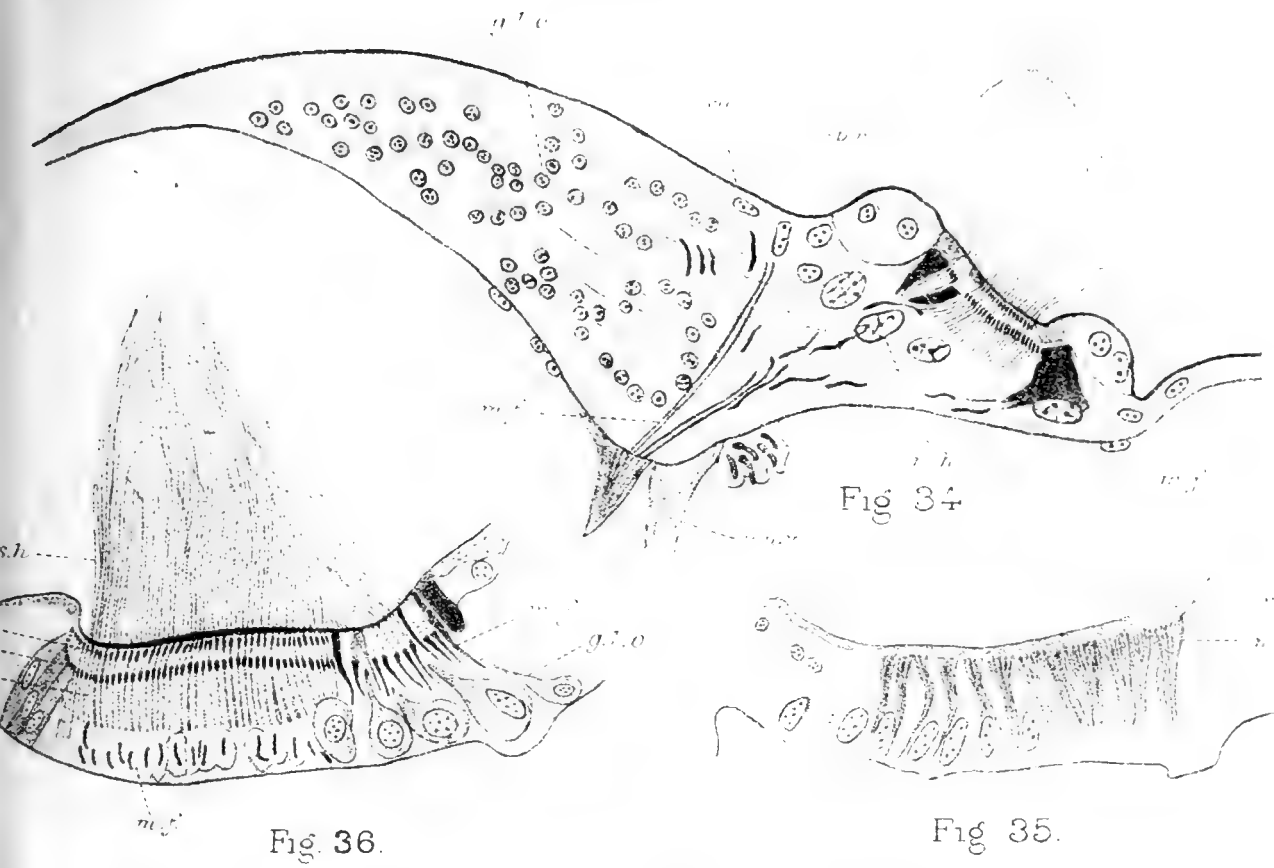
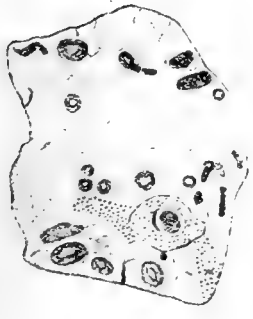


Fig. 34

Fig. 36.

Fig. 35.



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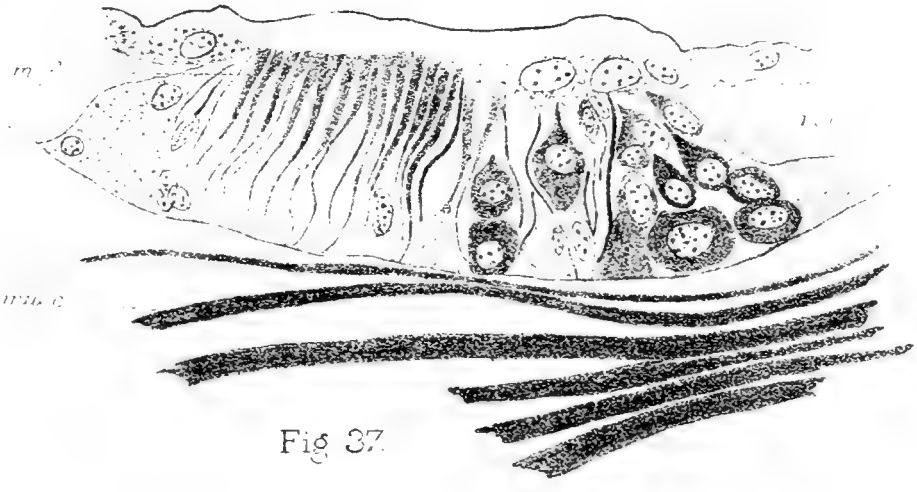

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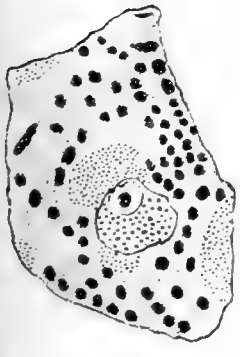

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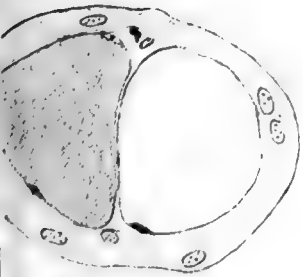
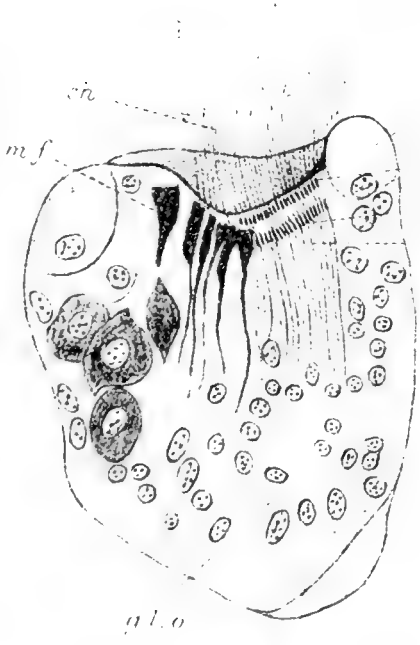
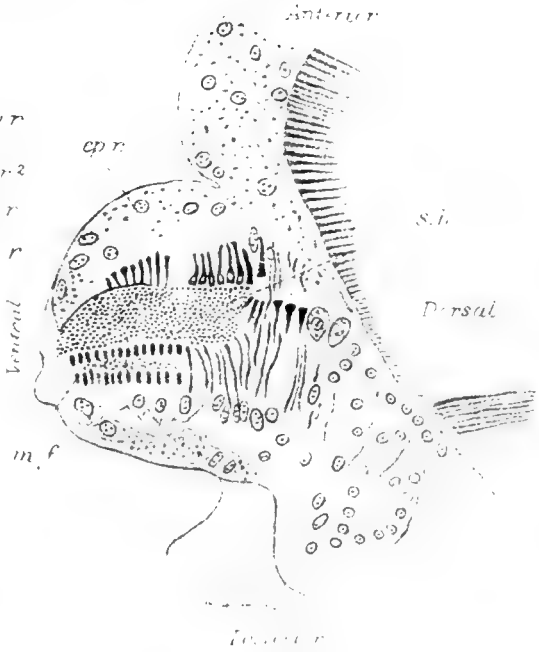






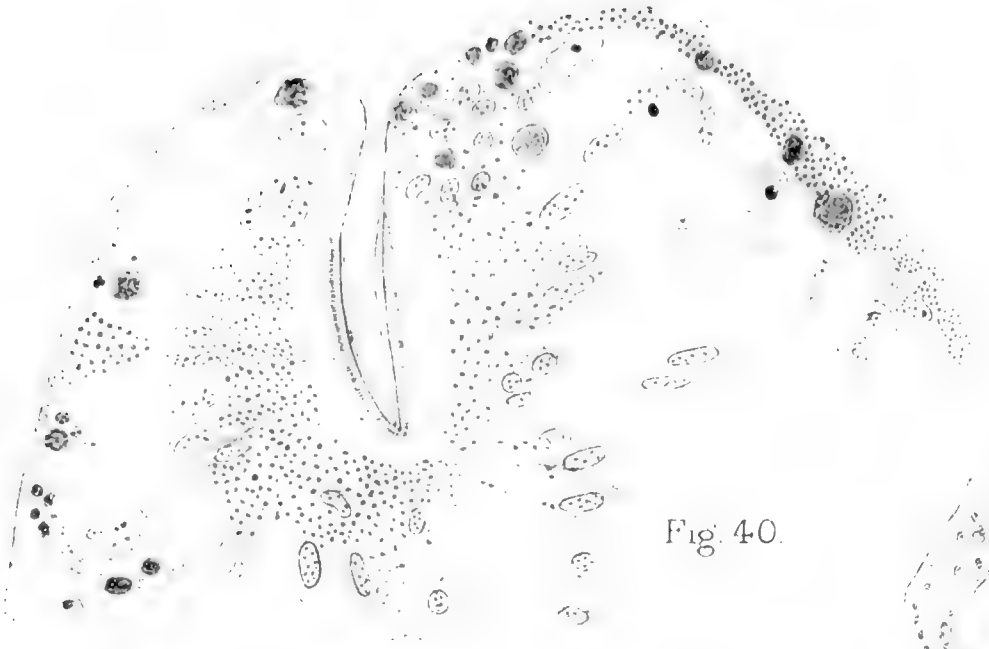
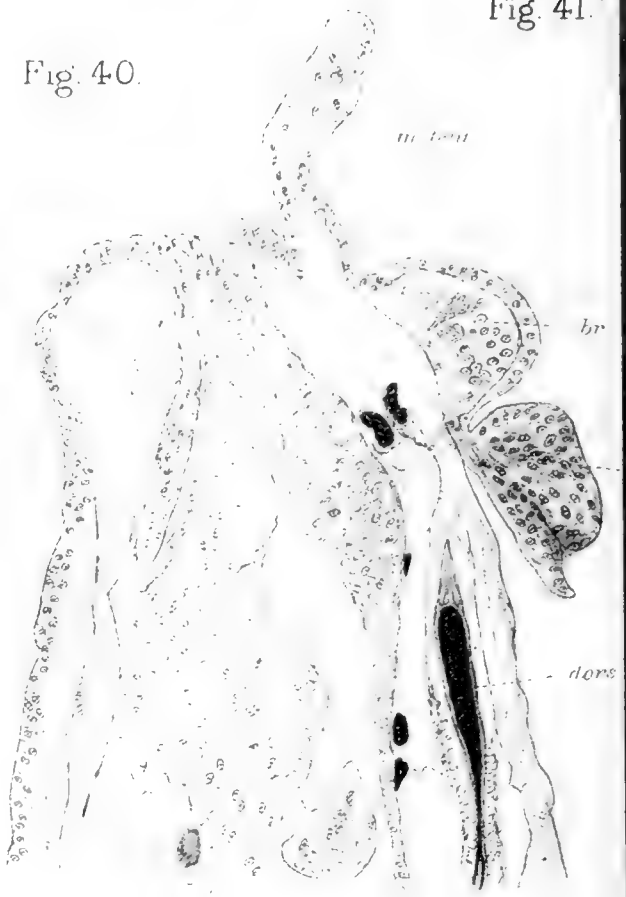


Fig. 40.

Fig. 41.



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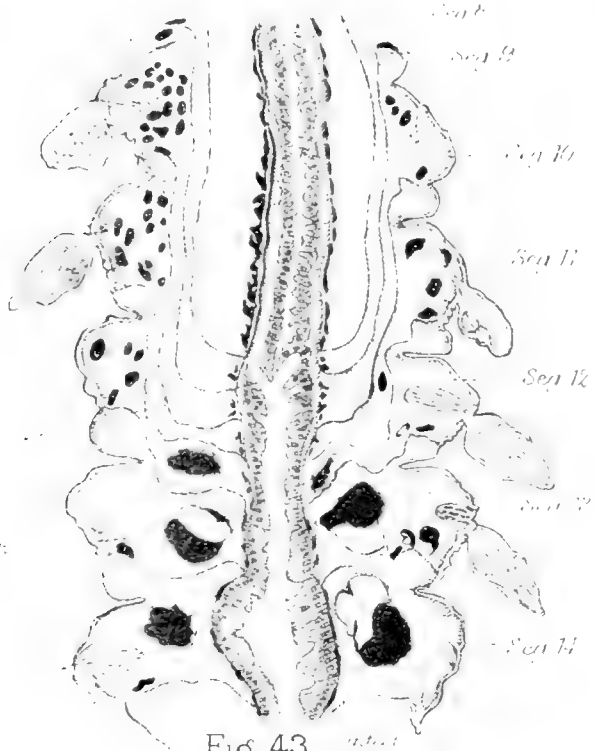
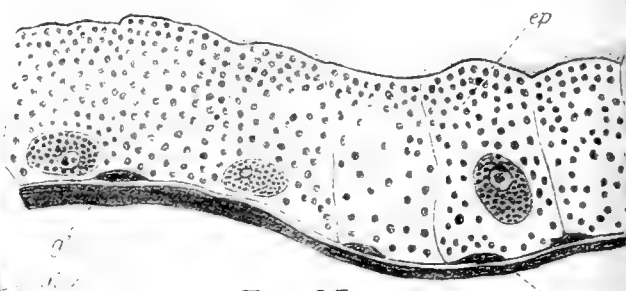


Fig. 43.



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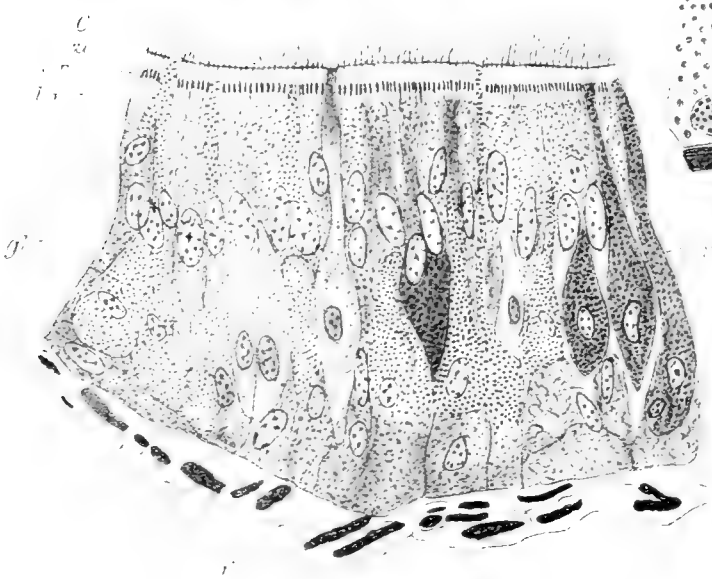

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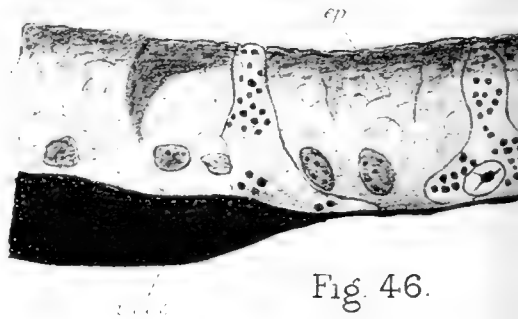

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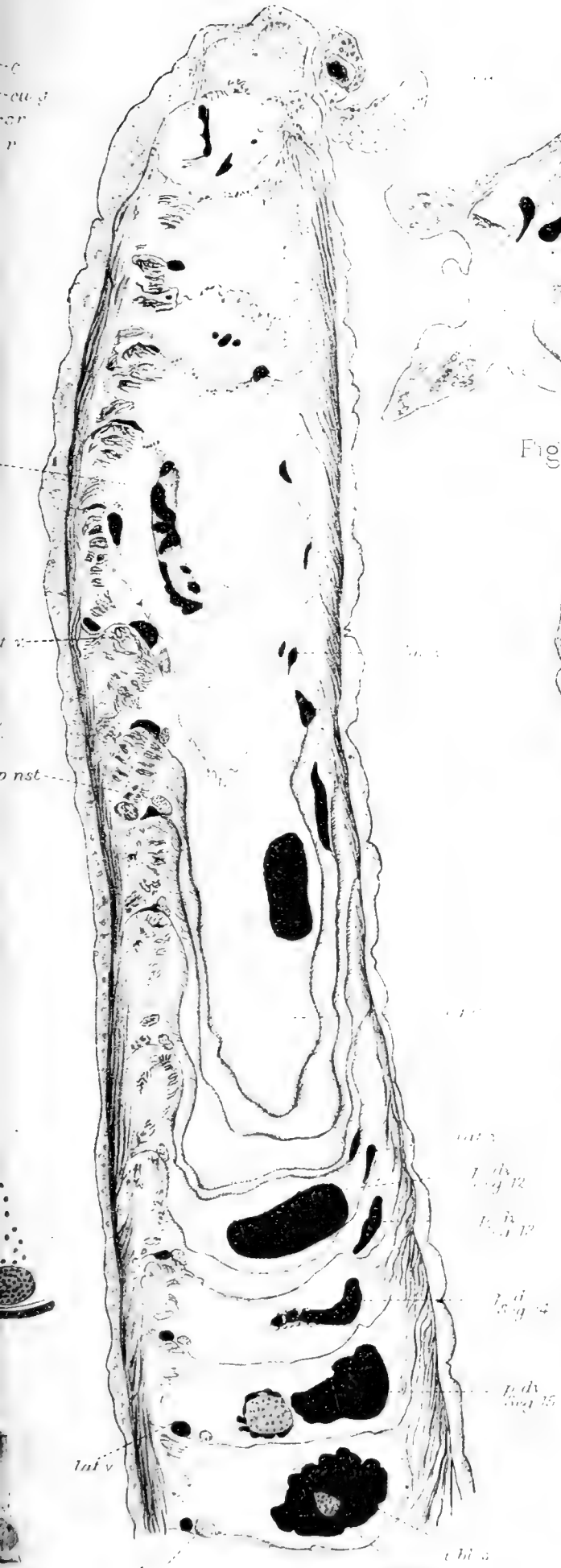
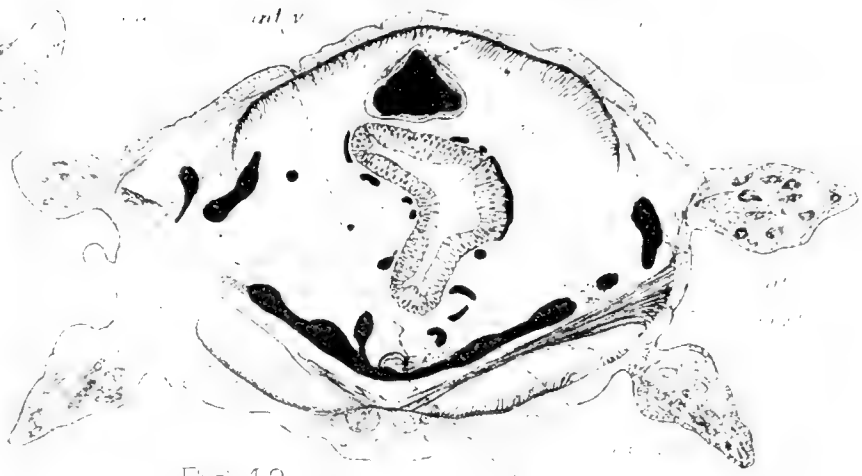


Fig. 47.



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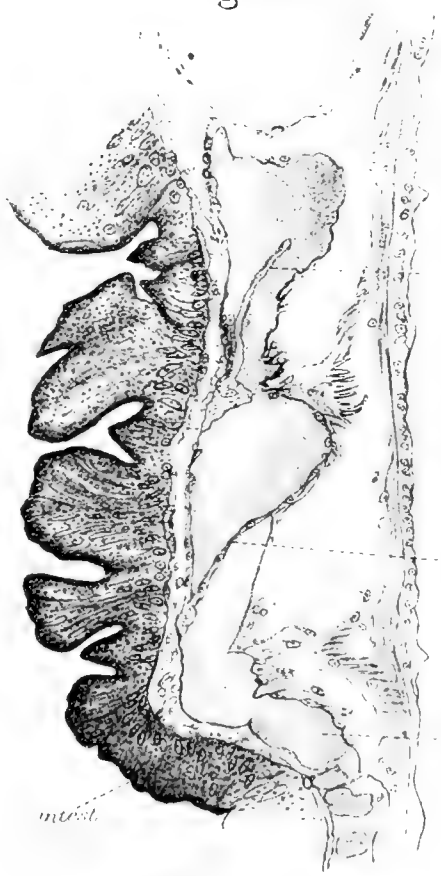

Fig. 50.

Fig 51.

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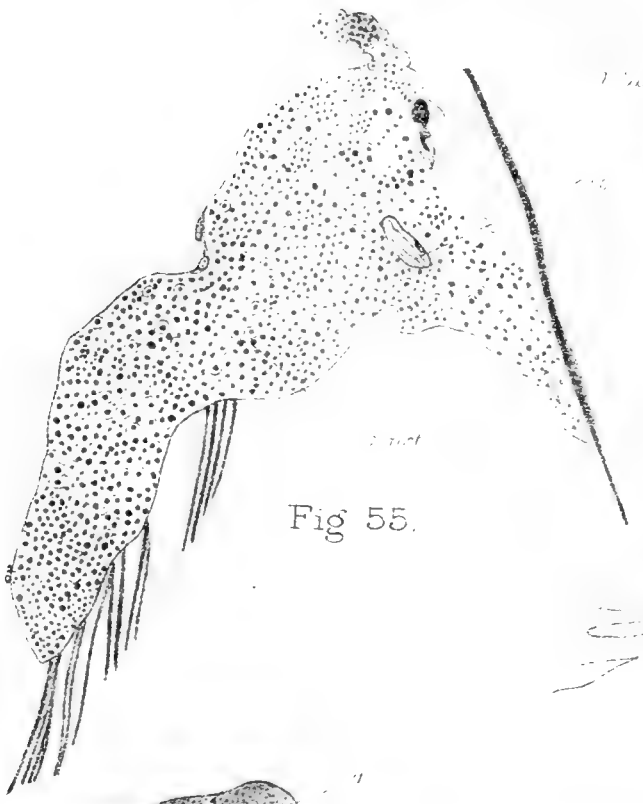
Fig. 53.



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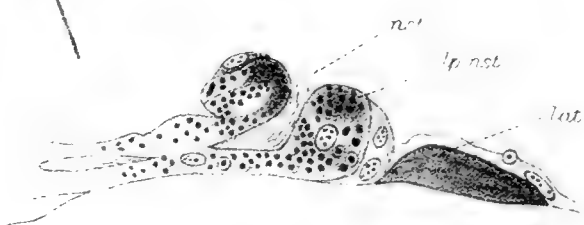

Fig. 54.

Fig. 57.

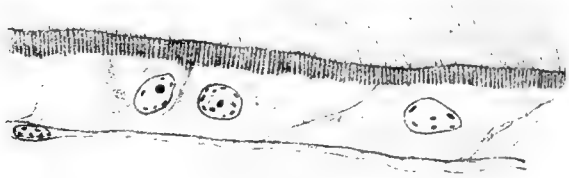

Fig. 56.

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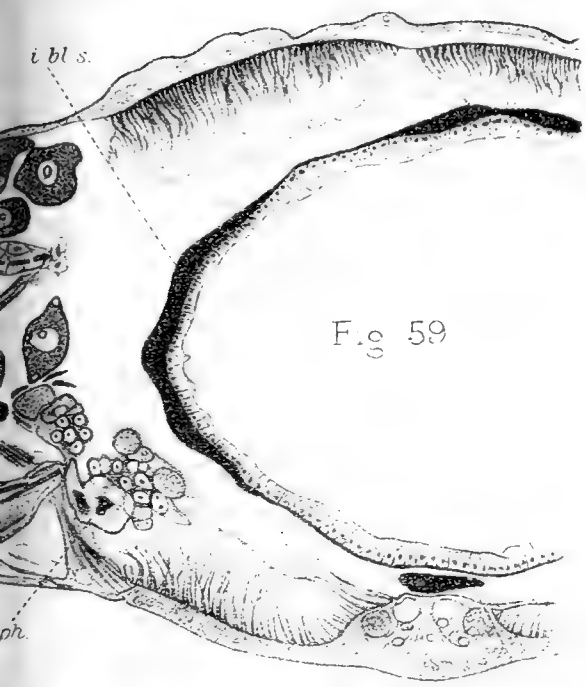


Fig. 59

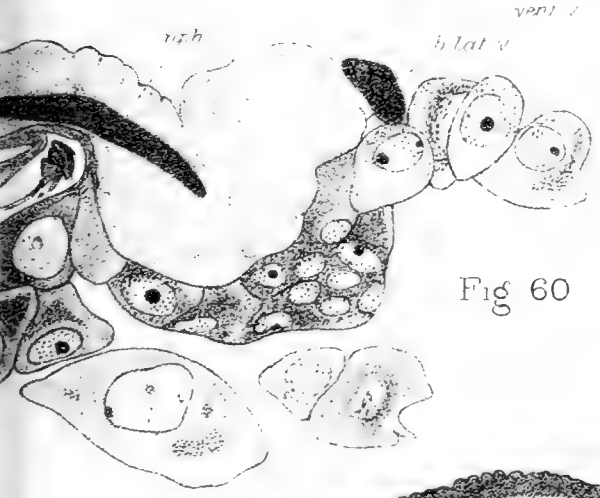
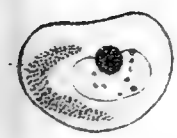


Fig. 60

Fig. 58.



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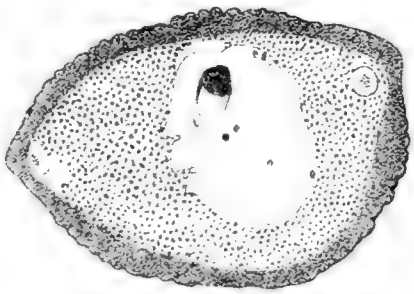

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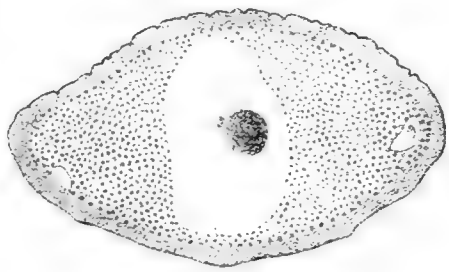


Fig. 63

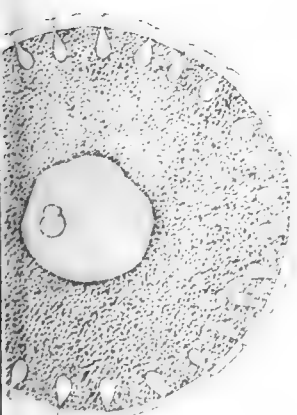


Fig. 64

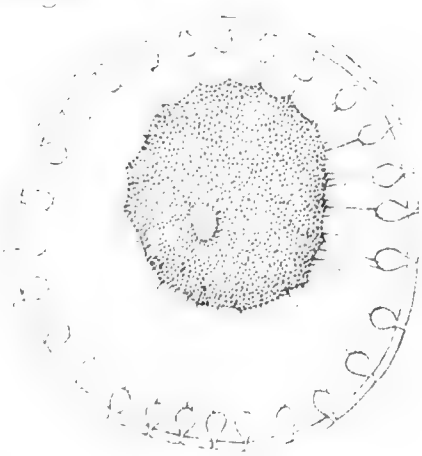


Fig. 65.

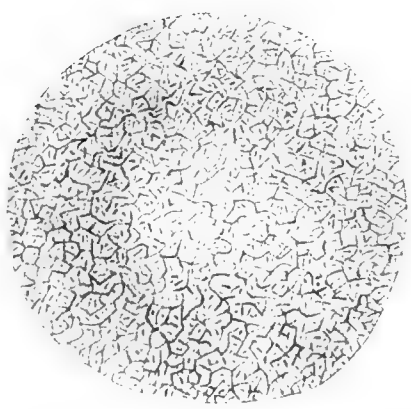


Fig. 66

Hutchinson, London

Notes on Sporozoa.

By

H. M. Woodcock, B.Sc.(Lond.).

I. On Klossiella muris gen. et spec. nov., Smith and Johnson, 1902.

Smith and Johnson (1) recently described a new Coccidian parasitic in the kidneys of the mouse (Mus musculus). The seat of the infection is the renal epithelium of the convoluted tubules of the cortex and of the visceral layer of Bowman's capsules. The enormously hypertrophied parasite-containing cells swell out into and completely occlude the lumen of the tubule, causing entire disorganisation of the affected tissue.

The diagnostic characters on which the new genus is based are as follows. The sporogonic cycle is characterised by the development of twelve to fourteen spherical spores, each about 16 μ by 13 μ, and containing thirty to thirty-four banana-shaped sporozoites. Another phase of the life-history was also met with. This is taken by Smith and Johnson to represent either schizogony or the formation of microgametocytes, but actually which of the two is left an open question. As a matter of fact, the authors' figures leave no doubt that the stage which they have described as sporogonic is nothing more nor less than merogony or schizogony, while the other part of the cycle is, in all probability, the commencement of gametocyte formation. As this new Coccidian presents certain very interesting features, I have thought it worth while to give a re-interpretation of Smith and Johnson's

clear and careful drawings, the real significance of which will be readily manifest on comparing them with the figures of another Coccidian, Caryotropha mesnilii, lately described by Siedlecki (2) from a Polychæte, Polymnia nebulosa, where it inhabits the testis. The name Klossiella muris may quite well be retained, at any rate until the parasite is re-discovered and the number of its genuine spores and sporozoites determined, since, notwithstanding the resemblance between the schizogonic phase in the two forms, the very different habitat, and important distinctions in the manner of formation of the microgametocytes already preclude us from uniting the two genera together.

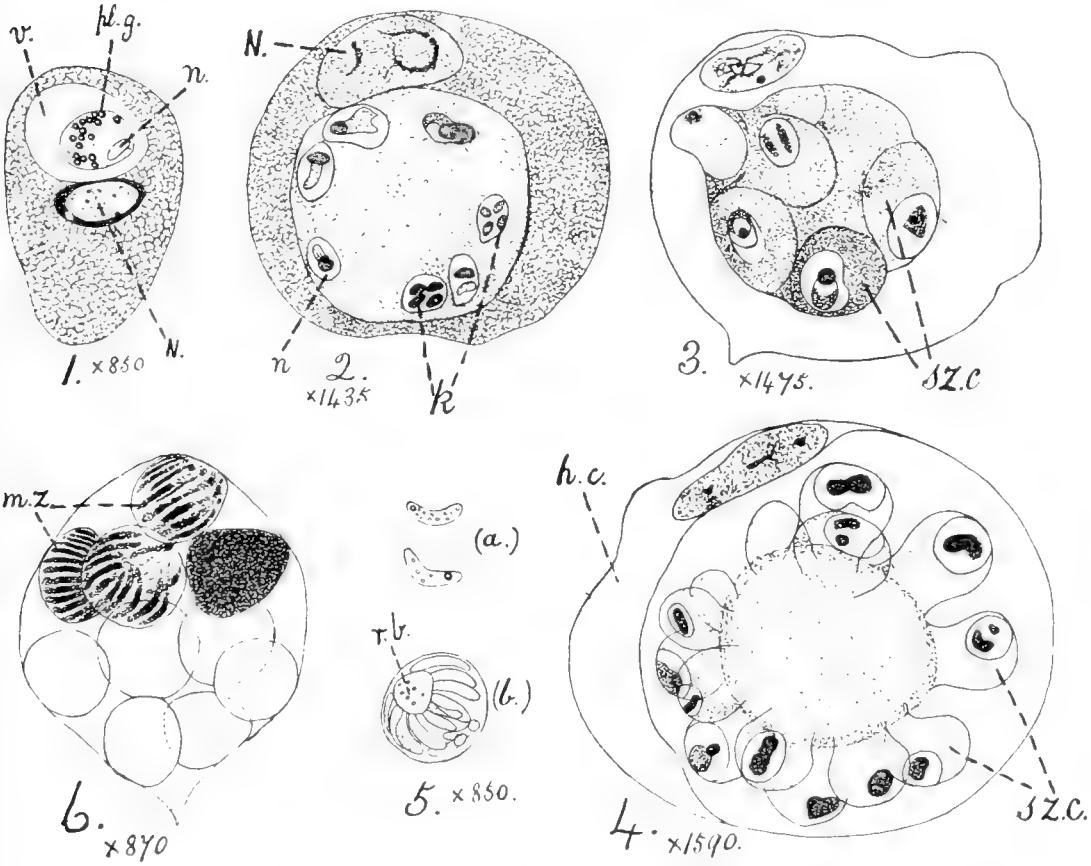
The drawings in Fig. *A* are reproduced from Smith and Johnson's figures, and those in Fig. *B* are copied from Siedlečki's paper. All are drawn the same size as the originals.[1] I will first give, as it were, a revised account of what is known of the life-history of Klossiella muris, and then proceed to justify my interpretation of the same, finally contrasting the genus with one or two other Coccidia. The authors' designations of the various stages are enclosed in square brackets.

In Fig. *A* (1) we have one of the smallest trophozoites [sporonts] seen. Such a young form, commencing to grow, is from 8 μ to 11 μ in diameter, and lies in a vacuole (*v.*) in the host-cell. Its membrane, so far as it has one, is very delicate, and practically only a limit to the cell. Each individual contains from ten to twenty plastin granules (*pl. g.*). "N." is the nucleus of the host-cell, and "n" that of the parasite. In the next figure, *A* (2), the trophozoite has become considerably larger (even allowing for the difference in magnification), and is now almost full-grown; it is, in fact, a schizont beginning merogony [mother-sporoblast]. Such an adult trophozoite or schizont

[1] A comparison of Smith and Johnson's different figures would have been greatly facilitated if they had been drawn to the same, or multiples of the same, magnification; while Siedlečki does not give the magnification of his at all.

may attain a diameter of as much as 40 μ. In the one before us the nucleus has already divided up into several, each possessing one to four karyosomes (*k.*), with usually a certain amount of granular chromatin besides. Around each of these daughter-nuclei the cytoplasm segregates itself, and thus the parasite becomes (superficially) divided up into a

Fig. A.



number of uninuclear portions (Fig. 3). These buds next commence to grow out at the periphery (Fig. 4), forming daughter-schizonts, or, as Siedlečki terms them, "schizontocytes" (*sz.c.*) [daughter-sporoblasts]. The host-cell is by this time greatly hypertrophied, and consists for the most part of a very delicate, attenuated layer of protoplasm, enclosing the huge vacuole in which the Klossiella lies; on one side (at *h. c.*) it is rather thicker, and this portion contains the nucleus, also much altered and hyperchromatosed. The schizontocytes

are at length cut off, and become separate inside the remains of the cell. According to Smith and Johnson, the central part of the cytoplasm of the mother-schizont may be entirely used up ("resorbed") by the daughter ones, as in Fig. *A* (6), or some may be left over as a residual body [restiform body]. In Fig. *A* (6) the contents of each separate schizontocyte [spore] have further divided up into a great number of merozoites (*m. z.*) [sporozoites], all arranged in one direction, and constituting, indeed, a typical merogonic "barillet." The homogeneous-looking masses are simply deeply stained daughter-schizonts, too opaque to show the merozoites inside. It will be observed that the only "membrane" holding the products resulting from one parasite together is the completely atrophied host-cell. Fig. *A* (5 *b*) shows a single barillet of merozoites liberated from a fresh kidney; the cluster is attached to a small secondary residual body (*r. b.*). Our authors state that the membrane surrounding the merozoites [i. e. the spore-membrane] is usually rounded, but of no definite shape and quite structureless, and in optical section appears only as a sharp line; moreover, it is easily ruptured on pressure, setting free the enclosed merozoites. In short, it doubtless represents, in its turn, the remains of the schizontocyte, nearly all of which has been used up to form the cluster. At (*a*) in the same figure are seen two free, unstained merozoites [sporozoites], each about 7 μ by 3 μ and containing several little vacuoles, one of which is often more prominent than the rest.

Such is the so-called sporogony of this Coccidian. With regard to the other phase of the life-history (Smith and Johnson's two figures of which I have not thought it necessary to reproduce) a few words will suffice at present, since it in no way affects the question of the sporogony of the phase above described. The authors term this the "glomerular" stage of the parasite, since it is found in the epithelium of Bowman's capsules, whereas the other form principally occurs in the convoluted tubules. As the glomerular form was only found in kidneys already infected with Klossiella, we can,

I think, agree with Smith and Johnson that the two are in some way related.

The chief difference between them is that in the former there is no "budding" nor anything analogous to the formation of schizontocytes. As the young parasites grow the (at first single) nucleus divides successively to form a great many, evenly distributed throughout the cytoplasm. The latter then segments up around these daughter-nuclei, and there result numerous "falciform bodies," which are, however, not nearly so sickle-shaped as the merozoites, but more of an elongated lozenge form. Each of them is about 7 μ by 2 μ, and possesses a rather small nucleus, centrally situated. The further history of these bodies was not followed; the authors suggest that the process may represent either schizogony or microgametocyte-formation, saying that the position is a favourable one for the development of either phase, but they do not decide between the two hypotheses, though perhaps, on the whole, rather inclined to support the latter. Nothing in the nature of macrogametocyte-formation was noticed.

I propose now to summarise my reasons, most of which will be, I think, already evident, for considering that the more fully-described part of the life-cycle of Klossiella is, in reality, only the schizogonous phase—serving for auto-reproduction, and not the sporogonic phase—producing resistant spores capable of transmitting the species to a fresh host. The spore-forming cyst, or oocyst, in the Coccidia is the result of fertilisation of a macrogamete by a microgamete, and may be looked upon as the final stage of the life-history undergone in the host. Representing, as it does, the termination of growth, the large macrogametocyte up to the time of maturation is contained within an atrophied host-cell, from whose shrunken and shrivelled remains it is set free prior to fertilisation. After conjugation (indeed in some cases before, e. g. in Coccidium proprium and C. faurei) a cyst-membrane is rapidly secreted round the oocyte (now the sporont), which becomes thick and tough and affords protection to the developing contents. Obviously, no further increase in size

is possible. Moreover the sporont is typically extra-cellular during the whole course of sporogony. Compare this with what we find in Klossiella. In Fig. *A* (1) we have a young form possessing, at most, a very delicate membrane, and lying in a vacuole in a host-cell that as yet shows hardly any effects of the parasitic invasion. Again, this young "sporont" grows from 10 μ to as much as 40 μ! Further, in the nuclei and nuclear division in a Coccidian sporont—in fact, while the sporogonic cycle lasts—there is no sign of karyosomes. When, as in C. proprium, these are retained in the ripe gametes and are thus present in the oocyte, they are invariably left behind in the residual cytoplasm of the latter and take no part in spore-formation; and even their retention up to this stage is unusual. The presence of karyosomatic nuclei is, in short, essentially a mark of schizogony, be it male, female, or indifferent in type; and it is a feature in the multiplicative stages before us (Fig. *A* 2, 3, 4). We will leave out of account the markedly peripheral origin of the "buds," —although peripheral budding is characteristic of endogenous reproduction,—since in polysporous types (Klossia, etc.) there is a tendency to a similar mode of origin of the sporoblasts, with the formation of a central "reliquat kystal." Let us pass on to the "spores" themselves. There is now no doubt about the occurrence of polyzoic spores; Cyclospora itself and the re-investigated Eucoccidium ("Benedenia") octopianum are instances of it,—so it is quite possible that, in these cases, there may also be a more or less "barillet"-like arrangement of the sporozoites, such as is often met with in merozoites.

Here, however, the resemblance between the bodies seen in Fig. *A* (6) and spores ceases. Besides the very important facts that they are not enclosed in a definite oocyst and are still within the host-cell (the former of which, at any rate, would be without analogy in the order), there is another reason why these bodies cannot be regarded as representing true spores. This is their varying and indefinite shape—or rather their shapelessness,—together with the extremely delicate nature

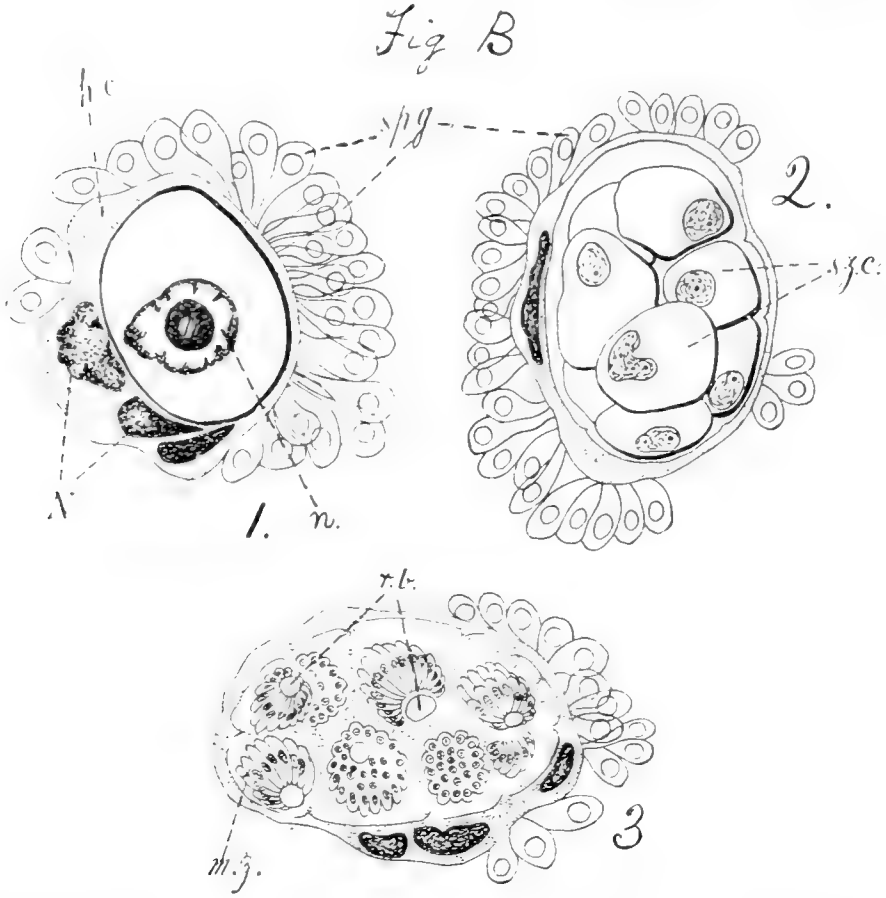
of the envelope enclosing each cluster of germs. A Coccidian sporocyst is always quite definite in form and fairly tough and resistant, and generally consists of two valves which separate under the action of the new host's digestive juices (sometimes, this can be effected artificially) to liberate the sporozoites. Nothing of the kind is mentioned in Smith and Johnson's account; the authors simply state that the membrane is very delicate, and easily ruptured on pressure. As I have above suggested, it much more probably represents (together with a small amount of residual material) the remains of a daughter-schizont, most of which has gone to form the merozoites. Between these and sporozoites, in the fresh condition, there is little difference, so that I need only add that if my interpretation is correct, the germs in Fig. *A* (5 *b*) belong to the former category and not to the latter.[1]

Of course the novel, and at that time unexampled variation which distinguishes schizogony in Klossiella from the usual method, might, to a certain extent, mislead the authors in interpreting their observations. Apart from this, however, the above-mentioned very characteristic facts relative to the general course of development of a Coccidian parasite and its relation to the host-cell ought to have rendered them suspicious in accepting the observed stages as constituting sporogony. As it happened Siedlečki (l. c.) very soon afterwards described a similar modification of merogony in Caryotropha. The resemblance between the process in the two genera is most striking, and I have above used this author's terminology in interpreting the phase as it occurs in Klossiella.

In Fig. *B* are reproduced some figures of Caryotropha for comparison with those in Fig. *A*. In (1) the host-cell (a spermatogonium) and two of its neighbours are greatly hypertrophied and have fused into a single mass containing

[1] Unfortunately it is impossible to tell from fig. 6 (the stained preparation) whether the germs have a karyosome in the nucleus or not, which would have conclusively settled the question.

the schizont. The cytoplasm of the parasite is left clear; its large karyosomatic nucleus is seen at (n), while at (N) we have the enlarged spermatogonial nuclei of the altered cells. "*Sp. g.*" represent normal spermatogonia around. The parasite, though not full grown, is, of course, relatively much



older than the young Klossiella schizont of Fig. *A* (1). The next stage of Caryotropha depicted, seen in Fig. *B* (2), shows a condition intermediate between Figs. *A* (4) and (6). The mother individual has divided up into daughter schizonts or schizontocytes, ten or more in number, which are separate, but have not yet commenced to form merozoites. From Siedlečki's account it is evident that these daughter-individuals have arisen in a manner perfectly analogous to their origin in Klossiella. He says that each of the nuclei resulting from the division of the original nucleus of the parasite pushes out at the surface of the body (surrounded,

doubtless, by a "bud" of cytoplasm), and between them deep grooves extend inwards, so that at length the whole schizont becomes cut up into several portions—the schizontocytes. He does not add whether any residual cytoplasm may be left over unused or not. A small point distinguishing the schizogony in this genus is the unusually minute size of the karyosomes, which are present in the daughter-nuclei only as one or two granules. I think the last doubt will be removed by a comparison of Figs. *B* (3) and *A* (6), especially if we regard each of the clusters in the latter as showing up like it does in Fig. 5 (*b*). In both cases all the "barillets" are enclosed by the partly or entirely atrophied cell or cell-mass, and by that alone. The only slight difference is that in those of Caryotropha the remains of the daughter-schizonts seem to have more completely broken down than they have in Klossiella, leaving no distinct enclosing membrane. It is, however, most likely that in older clusters of the latter genus the delicate investment around each also naturally breaks down, as, indeed, it must do if the essential object of schizogony, namely auto-infection, is to be attained.

The marked correspondence between the schizogonic process in the two forms does not appear to be maintained in microgametocyte-formation. In Caryotropha this resembles schizogony to a surprising extent, and serves to emphasize the complete homology of the two kinds of reproductive germ. Briefly stated, a number of microgametocytes of the second order (strictly comparable to schizontocytes) are intercalated between the original microgametocyte (of the first order) and the ultimate male gametes. The microgametes themselves arise from these daughter-microgametocytes exactly as if they originated in the usual manner from the microgametocyte of the first order, as, e. g. in Coccidium. Until ripe and ready for liberation they are all contained within the atrophied host-cell, just as are the clusters of merozoites. So far as can be gathered from Smith and Johnson's account nothing of the kind occurs in Klossiella; but this form, on the other hand, would appear to possess a

differentiation in another direction which is not met with in Caryotropha. In the latter there is no sign of an early differentiation of sexuality. The merozoites (representing the end term of schizogony), which grow into microgametocytes of the first order or macrogametocytes, respectively, are in no way different from the indifferent ones which become ordinary schizonts; that is to say, there is no male or female schizogony accompanied by the formation of male or female merozoites such as we find in certain cases (Adelea, Cyclospora). Now in Klossiella the "glomerular" form mentioned above almost certainly represents either male or female schizogony, leading to gametocyte-formation, and this view is supported by the authors' remark that the phase was only found in kidneys already strongly infected with the other stage, i. e. when merogony, we may assume, had almost run its potential course. In the absence of any further knowledge of the parasite it is impossible to say with certainty which sex the lozenge-shaped bodies above described represent; whether, in other words, they will grow into micro- or macrogametocytes. Smith and Johnson are inclined to think they may become the former, and suggest that they give rise to the actual gametes only when attached ("accolés") to a female element; they did not, however, observe this process taking place. Their shape somewhat recalls that of the male merozoites of A. mesnili as figured by Perez (3). Whether, if we accept these as male elements, the female merozoites (becoming macrogametocytes) are similar to the indifferent ones (as in A. mesnili, again), and whether they are formed in the same complicated manner, or by simple schizogony, are facts which have still to be ascertained. In any case the rediscovery of Klossiella muris, and the working out of its complete life-history, would probably furnish some very interesting and important additions to our knowledge of the Coccidia.

References.

1. Smith, T., and Johnson, H. P.—"On a Coccidium (Klossiella muris, gen. et sp. nov.), Parasitic in the Renal Epithelium of the Mouse," 'J. Exptl. Medicine, Baltimore,' vi, pp, 1—21, pls. 1—4 (1902).

2. Siedlečki, M.—"Cycle évolutif de la Caryotropha mesnilii, coccidie nouvelle des Polymnies: note préliminaire," 'Bull. Ac. Cracovie,' 1902, pp. 561—568, 5 text-figs.

3. Perez, C.—"Le cycle évolutif de l'Adelea mesnili," 'Arch. f. Protistenk,' ii, pp. 1—12, pl., 1 and 4 text-figs. (1903).

The Structure and Classification of the Arachnida.

By

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Arachnida is the name given in 1815 by Lamarck (Greek *ἀράχνη*, a spider) to a class which he instituted for the reception of the spiders, scorpions, and mites previously classified by Linnæus in the order Aptera of his great group Insecta. Lamarck at the same time founded the class Crustacea for the lobsters, crabs, and water-fleas, also until then included in the order Aptera of Linnæus. Lamarck included the Thysanura and the Myriapoda in his class Arachnida. The Insecta of Linnæus was a group exactly equivalent to the Arthropoda founded a hundred years later by Siebold and Stannius. It was thus reduced by Lamarck in area, and made to comprise only the six-legged, wing-bearing "Insecta." For these Lamarck proposed the name Hexapoda; but that name has been little used, and they have retained to this day the title of the much larger Linnæan group, viz. Insecta. The position of the Arachnida in the great subphylum Arthropoda, according to recent anatomical and embryological researches, is explained in another article (Arthropoda). The Arachnida form a distinct class or line of descent in the grade Euarthropoda, diverging (perhaps in common at the start with the Crustacea) from primitive Euarthropods, which gave rise also to the separate lines of

descent known as the classes Diplopoda, Crustacea, Chilopoda, and Hexapoda.

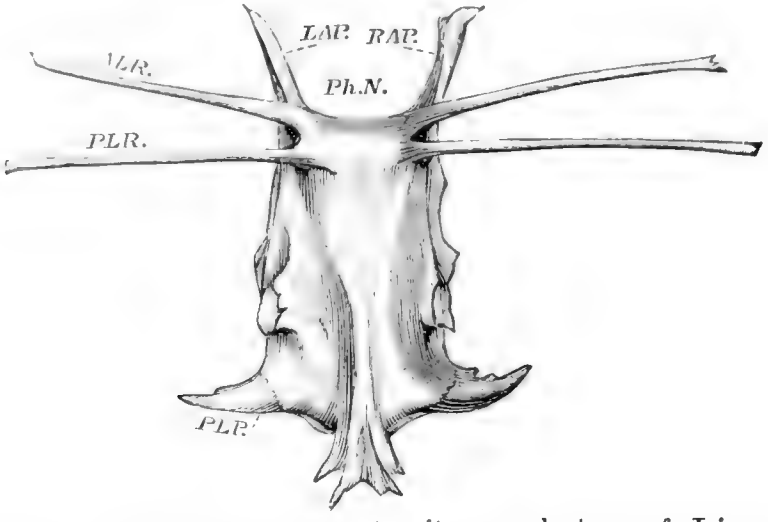


Fig. 1.—Entosternum, entosternite or plastron of Limulus polyphemus, Linn. Dorsal surface. *LAP*, left anterior process; *RAP*, right anterior process; *PhN*, pharyngeal notch; *ALR*, anterior lateral rod or tendon; *PLR*, posterior lateral rod or tendon; *PLP*, posterior lateral process. Natural size. (From Lankester, 'Q. J. Micr. Sci.,' N.S., vol. xxiv, 1884.)

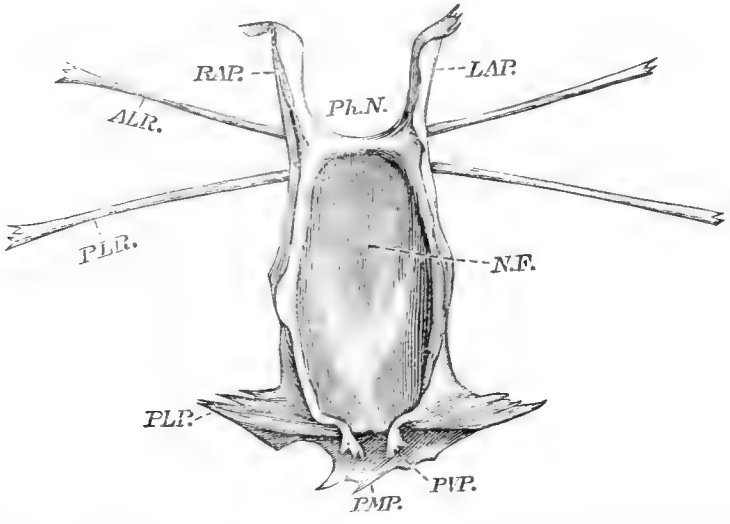


Fig. 2.—Ventral surface of the entosternum of Limulus polyphemus, Linn. Letters as in Fig. 1 with the addition of *NF*, neural fossa protecting the aggregated ganglia of the central nervous system; *PVP*, left posterior ventral process; *PMP*, posterior median process. Natural size. (From Lankester.)

Limulus an Arachnid.—Modern views as to the classification and affinities of the Arachnida have been determined

by the demonstration that Limulus and the extinct Eurypterines (Pterygotus, etc.) are Arachnida; that is to say, are identical in the structure and relation of so many important parts with Scorpio, whilst differing in those respects from other Arthropoda that it is impossible to suppose that the identity is due to homoplasy or convergence, and the con-

Fig. 3.

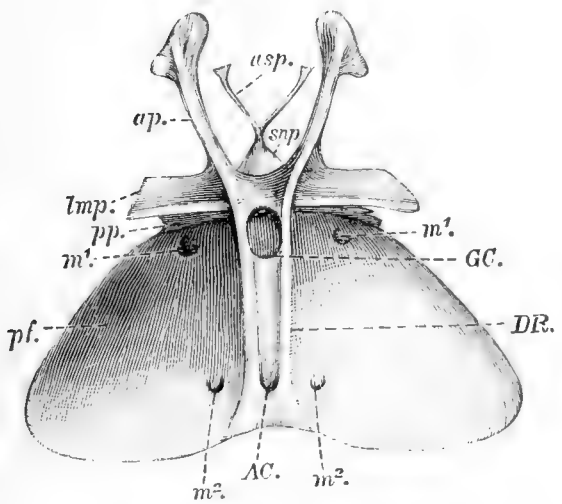


Fig. 4.

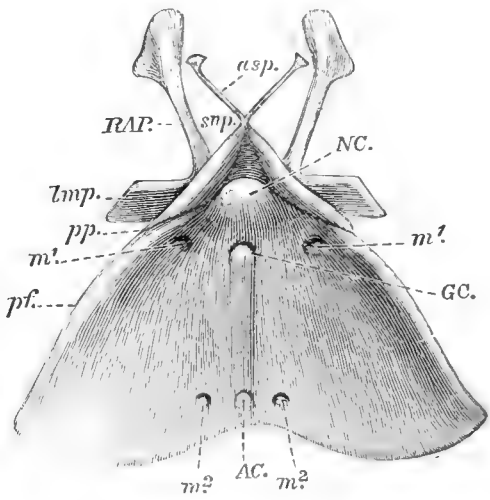


Fig. 3.—Entosternum of Scorpion (Palamnœus indus, De Geer); dorsal surface. *asp*, paired anterior process of the sub-neural arch; *snp*, sub-neural arch; *ap*, anterior lateral process (same as *RAP* and *LAP* in Fig. 1); *lmp*, lateral median process (same as *ALR* and *PLR* of Fig. 1); *pp*, posterior process (same as *PLP* in Fig. 1); *pf*, posterior flap or diaphragm of Newport; m^1 and m^2, perforations of the diaphragm for the passage of muscles; *DR*, the paired dorsal ridges; *GC*, gastric canal or foramen; *AC*, arterial canal or foramen. Magnified five times linear. (After Lankester, loc. cit.)

Fig. 4.—Ventral surface of the same entosternum as that drawn in Fig. 3. Letters as in Fig. 3 with the addition of *NC*, neural canal or foramen. (After Lankester, loc. cit.)

clusion must be accepted that the resemblances arise from close genetic relationship. The view that Limulus, the king-crab, is an Arachnid was maintained as long ago as 1829 by Straus-Durkheim (**1**), on the ground of its possession of an internal cartilaginous sternum—also possessed by the Arachnida (see Figs. 1—6),—and of the similarity of the disposition of the six leg-like appendages around the mouth in the two

cases (see Figs. 45 and 63). The evidence of the exact equivalence of the segmentation and appendages of Limulus and Scorpio, and of a number of remarkable points of agreement in their structure, was furnished by Lankester in an article published in 1881 ("Limulus an Arachnid," 'Quart. Journ. Micr. Sci.,' vol. xxi, N.S.), and in a series of subsequent memoirs, in which the structure of the entosternum, of the coxal glands, of the eyes, of the veno-pericardiac muscles,

Fig. 5.

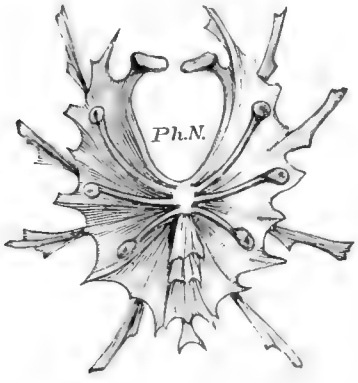


Fig. 6.

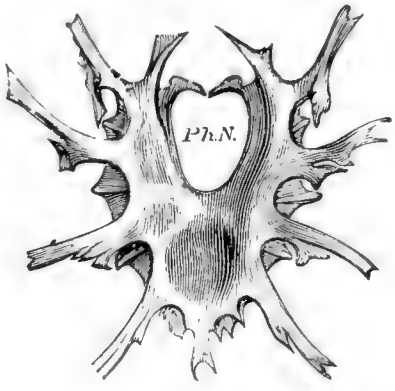


Fig. 5.—Entosternum of one of the mygalomorphous spiders; ventral surface. *Ph.N.*, pharyngeal notch. The three pairs of rod-like tendons correspond to the two similar pairs in Limulus, and the posterior median process with its repetition of triangular segments closely resembles the same process in Limulus. Magnified five times linear. (From Lankester, loc. cit.)

Fig. 6.—Dorsal surface of the same entosternum as that drawn in Fig. 5. *Ph.N.*, pharyngeal notch. (After Lankester, loc. cit.)

of the respiratory lamellæ, and of other parts, was for the first time described, and in which the new facts discovered were shown uniformly to support the hypothesis that Limulus is an Arachnid. A list of these memoirs is given at the close of this article (**2**, **3**, **4**, **5**, and **13**). The Eurypterines (Gigantostraca) were included in the identification, although at that time they were supposed to possess only five pairs of anterior or prosomatic appendages. They have now been shown to possess six pairs (Fig. 47), as do Limulus and Scorpio.

The various comparisons previously made between the

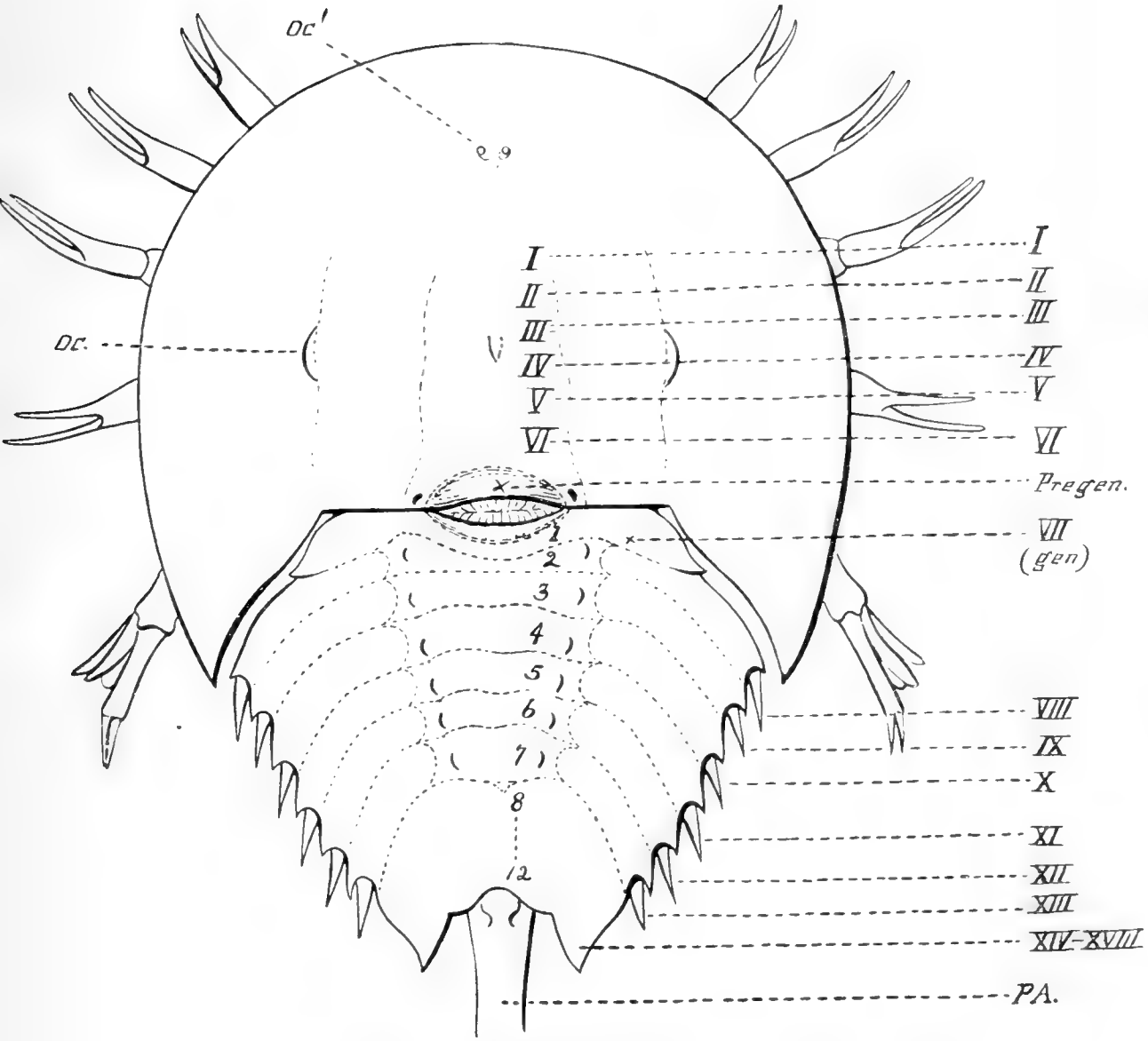
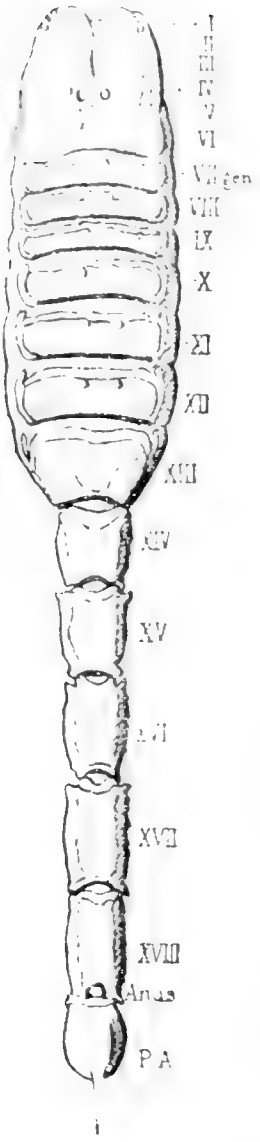


Fig. 7.—Diagram of the dorsal surface of Limulus polyphemus. *oc*, lateral compound eyes; *oc'*, central monomeniscous eyes; PA, post-anal spine; I to VI, the six appendage-bearing somites of the prosoma; VII, probably to be considered as the tergum of the genital somite; VII to XII, the six somites of the mesosoma; XIII to XVIII, the six somites of the metasoma, of which the first (marked XIII at the side and 7 on the tergum) is provided with a lateral spine, and is separated by ridges from the more completely fused five hinder somites lettered 8 to 12.

[This is a new figure replacing the Fig. 7 given in the 'Encyclopædia. It is at present a matter for further investigation as to whether the prægenital somite is merely represented by the piece marked × at the hinder border of the prosoma, or whether the area marked VII is the tergum of the prægenital somite, and that marked VIII the tergum of the genital somite. The disposition of the muscles and of the entopophyses should, when carefully studied, be sufficient to settle this point.—E. R. L.]

Fig. 8.



structure of Limulus and the Eurypterines on the one hand, and that of a typical Arachnid, such as Scorpio, on the other, had been vitiated by erroneous notions as to the origin of the nerves supplying the anterior appendages of Limulus (which were finally removed by Alphonse Milne-Edwards in his beautiful memoir [6] on the structure of that animal), and secondly by the erroneous identification of the double

Fig. 9.

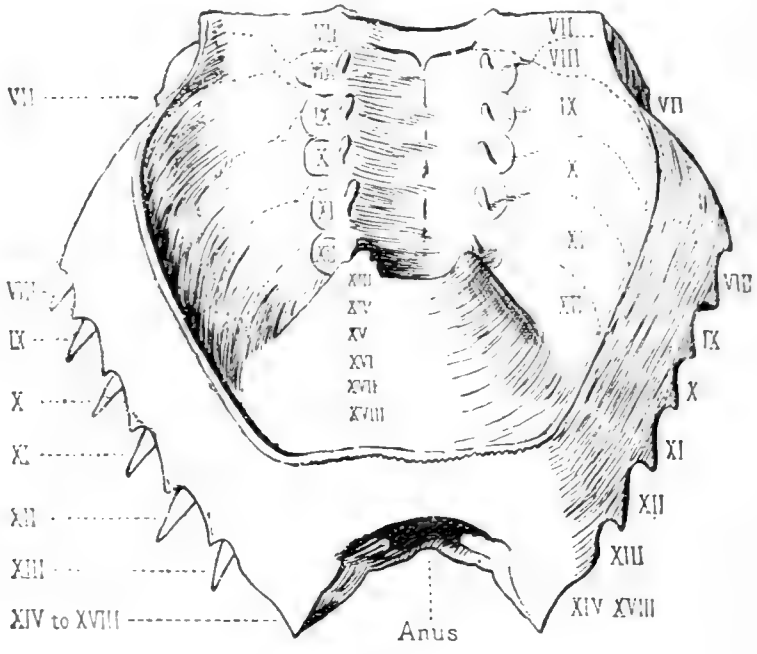


Fig. 8.—Diagram of the dorsal surface of a Scorpion to compare with Fig. 7. Letters and Roman numerals as in Fig. 7, excepting that VII is here certainly the tergum of the first somite of the mesosoma—the genital somite—and is not a survival of the embryonic prægenital somite. (From Lankester, loc. cit.) The anus (not seen) is on the sternal surface.

Fig. 9.—Ventral view of the posterior carapace or meso-metasomatic (opisthosomatic) fusion of Limulus polyphemus. The soft integument and limbs of the mesosoma have been removed as well as all the viscera and muscles, so that the inner surface of the terga of these somites with their entopophyses are seen. The unsegmented dense chitinous, sternal plate of the metasoma (XIII to XVIII) is not removed. Letters as in Fig. 7. (After Lankester, loc. cit.)

sternal plates of Limulus, called "chilaria" by Owen, with a pair of appendages (**7**). Once the identity of the chilaria with the pentagonal sternal plate of the scorpion is recognised — an identification first insisted on by Lankester—the whole series of segments and appendages in the two animals, Limulus and Scorpio, are seen to correspond most closely, segment for segment, with one another (see Figs. 7 and 8).

Fig. 10.

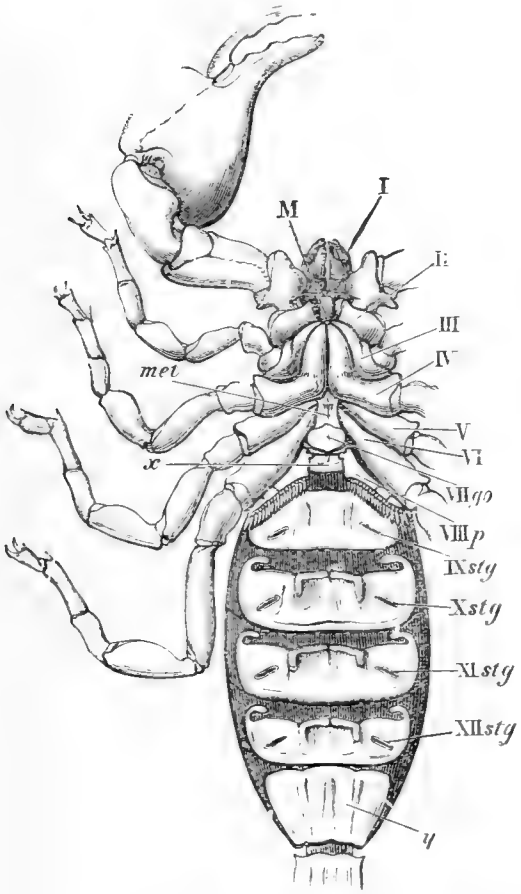


Fig. 11.

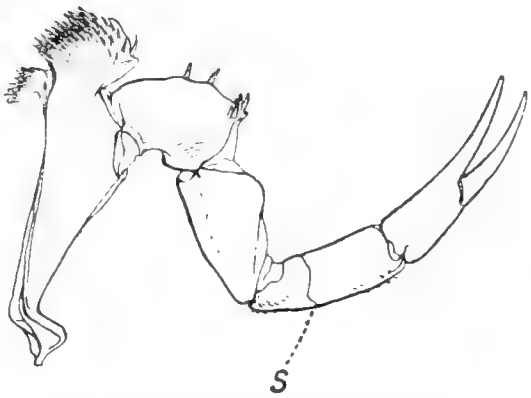


Fig. 10.—Ventral view of a Scorpion, Palamnæus indus, De Geer, to show the arrangement of the coxæ of the limbs, the sternal elements, genital plate and pectens. M, mouth behind the oval median camerostome; I, the cheliceræ; II, the chelæ; III to VI, the four pairs of walking legs; VII*go*, the genital somite or first somite of the mesosoma with the genital operculum (a fused pair of limbs); VIII*p*, the pectiniferous somite; IX*stg* to XII*stg*, the four pulmonary somites; *met*, the pentagonal metasternite of the prosoma behind all the coxæ; *x*, the sternum of the pectiniferous somite; *y*, the broad first somite of the metasoma.

Fig. 11.—Third leg of Limulus polyphemus, showing the division of the fourth segment of the leg by a groove *S* into two, thus giving seven segments to the leg as in Scorpion. (From a drawing by Mr. Pocock.)

The structure of the prosomatic appendages or legs is also seen to present many significant points of agreement (see

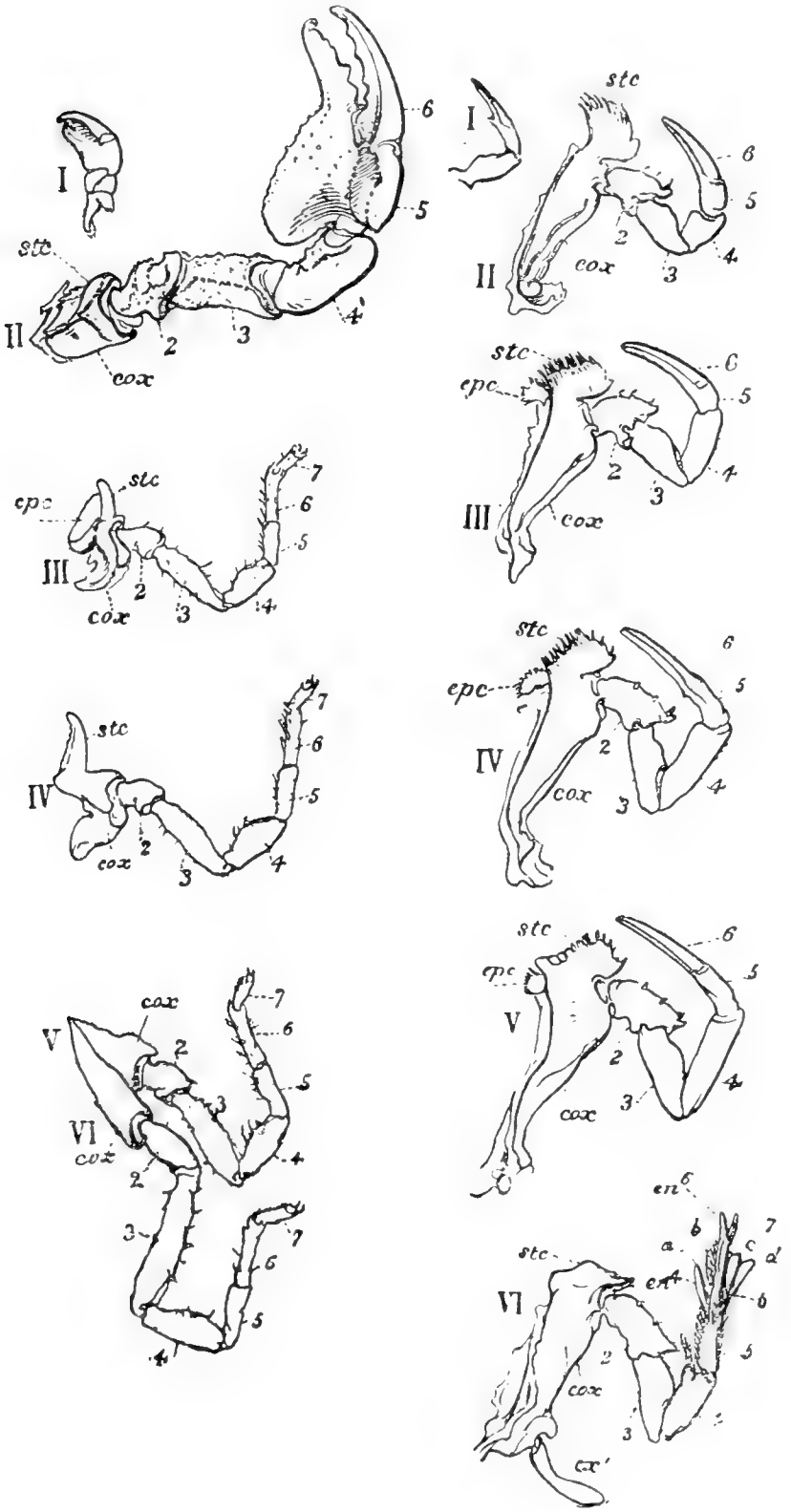
Figures), but a curious discrepancy existed in the six-jointed structure of the limb in Limulus, which differed from the seven-jointed limb of Scorpio by the defect of one joint. Mr. R. I. Pocock, of the British Museum, has lately observed that in Limulus a marking exists on the fourth joint, which apparently indicates a previous division of this segment into two, and thus establishes the agreement of Limulus and Scorpio in this small feature of the number of segments in the legs (see Fig. 11).

It is not desirable to occupy the limited space of this article by a full description of the limbs and segments of Limulus and Scorpio. The reader is referred to the complete series of figures here given, with their explanatory legends (Figs. 12—15). Certain matters, however, require comment and explanation to render the comparison intelligible.[1] The tergites, or chitinised dorsal halves of the body rings are fused to form a "prosomatic carapace," or carapace of the prosoma, in both Limulus and Scorpio (see Figs. 7 and 8). This region corresponds in both cases to six somites, as indicated by the presence of six pairs of limbs. On the surface of the carapace there are in both animals a pair of central eyes with simple lens and a pair of lateral eye-tracts, which in Limulus consist of closely aggregated simple eyes, forming a "compound" eye, whilst in Scorpio they present

[1] The discussion of the segmentation or metamerism of the Arachnida in this article should be read after a perusal of the article ARTHROPODA by the same author ('Q. Journ. Micr. Sci.,' vol. xlvii, n. s. p. 523).

FIG. 12.—The prosomatic appendages of Limulus polyphemus (right) and Scorpio (left), Palamnæus indus compared. The corresponding appendages are marked with the same Roman numeral. The Arabic numerals indicate the segments of the legs. *cox*, coxa or basal segment of the leg; *stc*, the sterno-coxal process or jaw-like upgrowth of the coxa; *epc*, the articulated movable outgrowth of the coxa, called the epicoxite (present only in III of the Scorpion and III, IV, and V of Limulus); ex^1, the exopodite of the sixth limb of Limulus; *a*, *b*, *c*, *d*, movable processes on the same leg (see for some suggestions on the morphology of this leg, Pocock in 'Quart. Journ. Micr. Sci.,' March, 1901; see also Fig. 50 on p. 235 and explanation). (From Lankester, loc. cit.)

Fig. 12.



several separate small eyes. The microscopic structure of the central and the lateral eyes has been shown by Lankester and Bourne (5) to differ; but the lateral eyes of Scorpio were shown by them to be similar in structure to the lateral eyes of Limulus, and the central eyes of Scorpio to be identical in structure with the central eyes of Limulus (see pp. 182, 183).

Following the prosoma is a region consisting of six segments (Figs. 14 and 15), each carrying a pair of plate-like appendages in both Limulus and Scorpio. This region is called the mesosoma. The tergites of this region and those

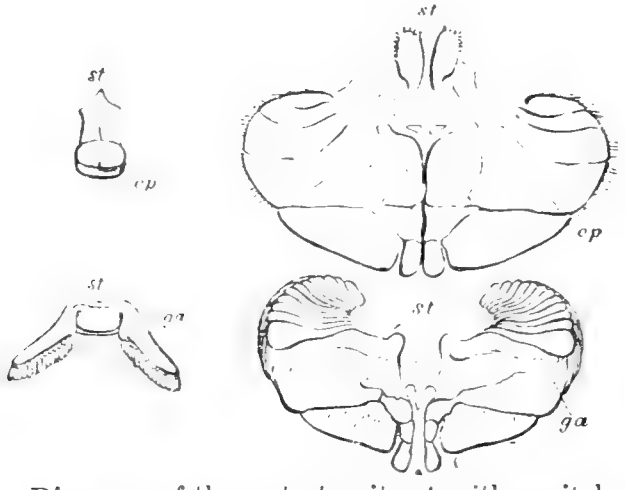


FIG. 13.—Diagrams of the metasternite *st*, with genital operculum *op*, and the first lamelligerous pair of appendages *ga*, with uniting sternal element *st* of Scorpio (left) and Limulus (right). (From Lankester, loc. cit.)

of the following region, the metasoma, are fused to form a second or posterior carapace in Limulus, whilst remaining free in Scorpio. The first pair of foliaceous appendages in each animal is the genital operculum; beneath it are found the openings of the genital ducts. The second pair of mesosomatic appendages in Scorpio are known as the "pectens." Each consists of an axis, bearing numerous blunt tooth-like processes arranged in a series. This is represented in Limulus by the first gill-bearing appendage. The leaves (some 150 in number) of the gill-book (see figure) correspond to the tooth-like processes of the pectens of Scorpio. The

next four pairs of appendages (completing the mesosomatic series of six) consist, in both Scorpio and Limulus, of a base carrying each 130 to 150 blood-holding, leaf-like plates, lying on one another like the leaves of a book. Their minute structure is closely similar in the two cases; the leaf-like plates receive blood from the great sternal sinus, and serve

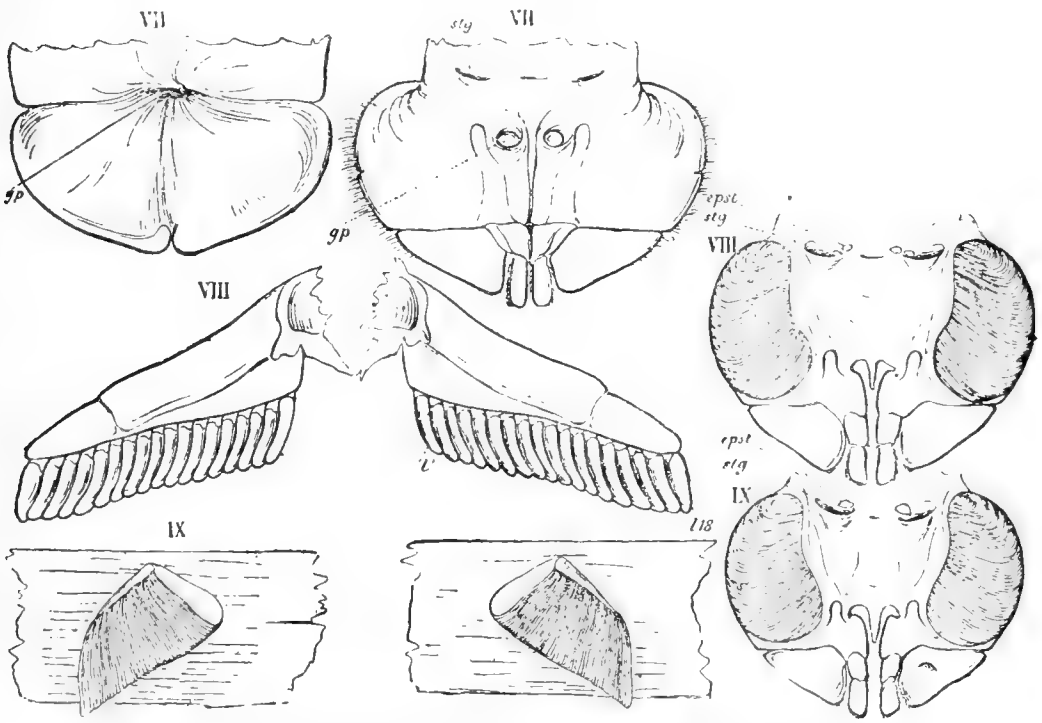


FIG. 14.—The first three pairs of mesosomatic appendages of Scorpio and Limulus compared. VII, the genital operculum; VIII, the pectens of Scorpio and the first branchial plate of Limulus; IX, the first pair of lung-books of Scorpio and the second branchial plate of Limulus; *gp*, genital pore; *epst*, epistimatic sclerite; *stg*, stigma or orifice of the hollow tendons of the branchial plates of Limulus. (After Lankester, loc. cit.)

as respiratory organs. The difference between the gill-books of Limulus and the lung-books of Scorpio depends on the fact that the latter are adapted to aërial respiration, while the former serve for aquatic respiration. The appendage carrying the gill-book stands out on the surface of the body in Limulus, and has other portions developed besides the gill-book and its base; it is fused with its fellow of the

opposite side. On the other hand, in Scorpio the gill-book-bearing appendage has sunk below the surface, forming a recess or chamber for itself, which communicates with the exterior by an oval or circular "stigma" (Fig. 10, *stg.*). That this in-sinking has taken place, and that the lung-books or in-sunken gill-books of Scorpio really represent appendages (that is to say, limbs or parapodia), is proved by their develop-

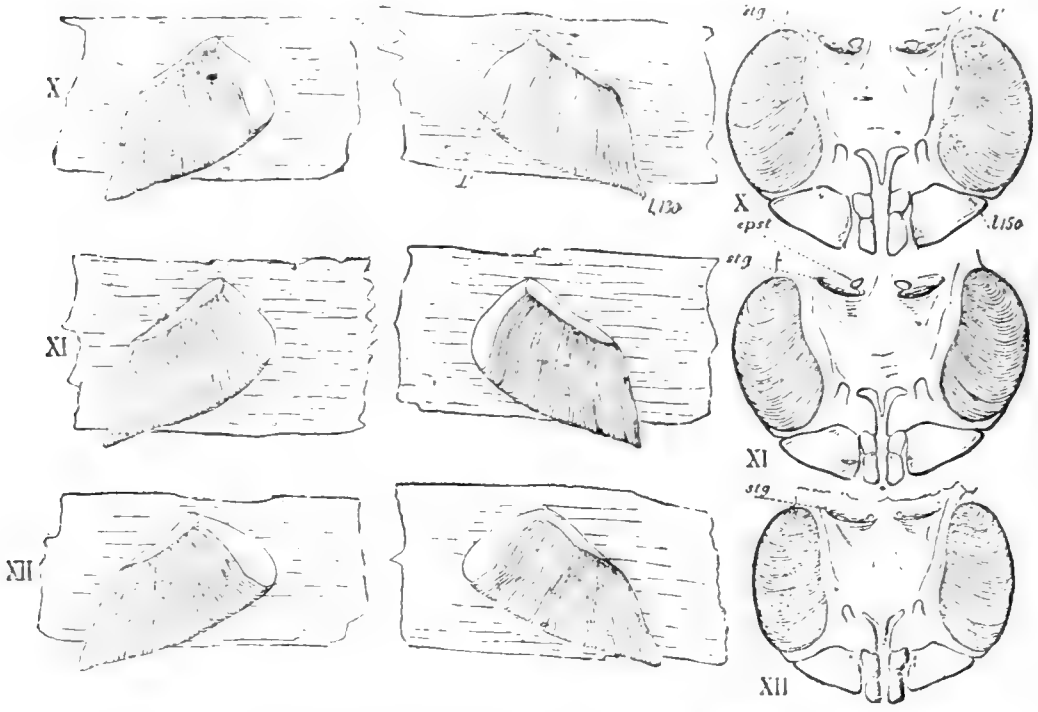


FIG. 15.—The remaining three pairs of mesosomatic appendages of Scorpio and Limulus. Letters as in Fig. 14. *l* 130 indicates that there are 130 lamellæ in the Scorpion's lung-book, whilst *l* 150 indicates that 150 similar lamellæ are counted in the gill of Limulus. (After Lankester, loc. cit.)

mental history (see Figs. 17 and 18). They appear at first as outstanding processes on the surface of the body.

The exact mode in which the in-sinking of superficial outstanding limbs, carrying gill-lamellæ, has historically taken place has been a matter of much speculation. It was to be hoped that the specimen of the Silurian scorpion (Palæophonus) from Scotland, showing the ventral surface of the mesosoma (Fig. 49), would throw light on this matter; but

the specimen, recently carefully studied by the writer and Mr. Pocock, reveals neither gill-bearing limbs nor stigmata. The probability appears to be against an actual introversion of the appendage and its lamellæ, as was at one time suggested by Lankester. It is probable that such an in-sinking as is shown in the accompanying diagram has taken

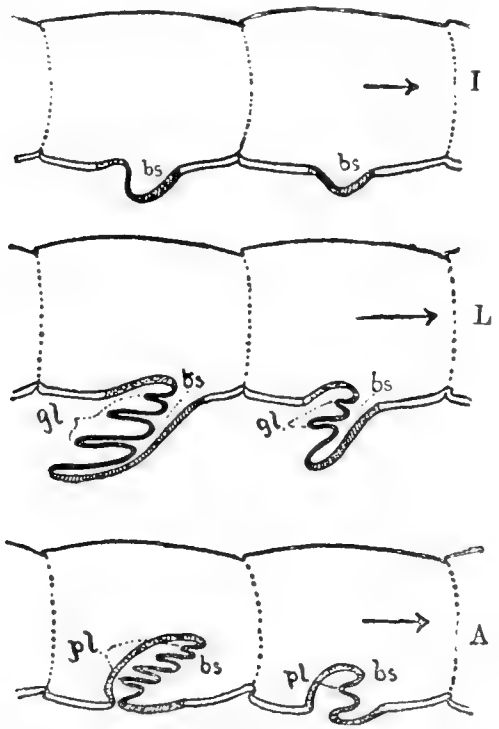


FIG. 16.—Diagram to show the way in which an outgrowing gill-process bearing blood-holding lamellæ may give rise, if the sternal body-wall sinks inwards, to a lung-chamber with air-holding lamellæ. I is the embryonic condition; *bs*, blood sinus; L is the condition of outgrowth with *gl*, gill lamellæ; A is the condition of in-sinking of the sternal surface and consequent enclosure of the lamelligerous surface of the appendage in a chamber with narrow orifice—the pulmonary air-holding chamber; *pl*, pulmonary lamellæ; *bs*, blood sinus. (After Kingsley.)

place (Fig. 16); but we are yet in need of evidence as to the exact equivalence of margins, axis, etc., obtaining between the lung-book of Scorpio and the gill-book of Limulus. Zoologists are familiar with many instances (fishes, crustaceans) in which the protective walls of a water-breathing organ or gill apparatus become converted into an air-breath-

ing organ or lung, but there is no other case known of the conversion of gill processes themselves into air-breathing plates.

The identification of the lung-books of Scorpio with the gill-books of Limulus is practically settled by the existence

Fig. 17.

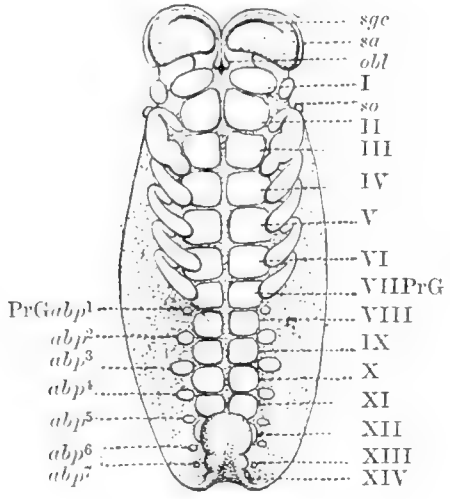


Fig. 18.

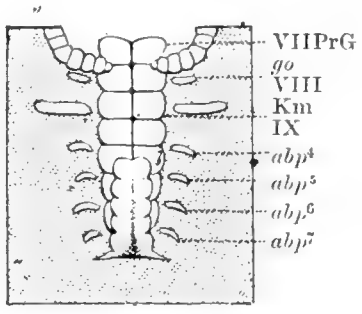


Fig. 17.—Embryo of Scorpion, ventral view showing somites and appendages. *sgc*, frontal groove; *sa*, rudiment of lateral eyes; *obl*, camerostome (upper lip); *so*, sense-organ of Patten; PrG*apb*^{1}, rudiment of the appendage of the prægenital somite which disappears; *abp*^{2}, rudiment of the right half of the genital operculum; *abp*^{3}, rudiment of the right pecten; *abp*^{4} to *abp*^{7}, rudiments of the four appendages which carry the pulmonary lamellæ; I to VI, rudiments of the six limbs of the prosoma; VIIPrG, the evanescent prægenital somite; VIII, the first mesosomatic somite or genital somite; IX, the second mesosomatic somite or pectiniferous somite; X to XIII, the four pulmoniferous somites; XIV, the first metasomatic somite. (After Brauer, 'Zeitsch. wiss. Zool.,' vol. lix, 1895.)

Fig. 18.—Portion of a similar embryo at a later stage of growth. The prægenital somite, VIIPrG, is still present, but has lost its rudimentary appendages; *go*, the genital operculum, left half; Km, the left pecten; *abp*^{4} to *abp*^{7}, the rudimentary appendages of the lung-sacs. (After Brauer, loc. cit.)

of the pectens in Scorpio (Fig. 14, VIII) on the second mesosomatic somite. There is no doubt that these are parapodial or limb appendages, carrying numerous imbricated secondary processes, and therefore comparable in essential structure to the leaf-bearing plates of the second mesosomatic somite of

Limulus. They have remained unenclosed and projecting on the surface of the body, as once were the appendages of the four following somites. But they have lost their respiratory function. In non-aquatic life such an unprotected organ cannot subserve respiration. The "pectens" have become more firmly chitinised and probably somewhat altered in shape as compared with their condition in the aquatic ancestral scorpions. Their present function in scorpions is not ascertained. They are not specially sensitive under ordinary conditions, and may be touched or even pinched without causing any discomfort to the scorpion. It is probable that they acquire special sensibility at the breeding season, and serve as "guides" in copulation. The shape of the legs and the absence of paired terminal claws in the Silurian Palæophonus (see Figs. 48 and 49) as compared with living scorpions (see Fig. 10) show that the early scorpions were aquatic, and we may hope some day, in better preserved specimens than the two as yet discovered, to find the respiratory organs of those creatures in the condition of projecting appendages serving aquatic respiration somewhat as in Limulus, though not necessarily repeating the exact form of the broad plates of Limulus.

It is important to note that the series of lamellæ of the lung-book and the gill-book correspond exactly in structure, the narrow, flat blood-space in the lamellæ being interrupted by pillar-like junctions of the two surfaces in both cases (see Lankester [4]), and the free surfaces of the adjacent lamellæ being covered with a very delicate chitinous cuticle which is drawn out into delicate hairs and processes. The elongated axis which opens at the stigma in Scorpio, and which can be cleared of soft surrounding tissues and coagulated blood so as to present the appearance of a limb axis carrying the book-like leaves of the lung, is not really, as it would seem to be at first sight, the limb axis. That is necessarily a blood-holding structure, and is obliterated and fused with soft tissues of the sternal region, so that the lamellæ cannot be detached and presented as standing out from it. The apparent axis or

basal support of the scorpion's lung-books shown in the figures is a false or secondary axis, and merely a part of the infolded surface which forms the air-chamber. The maceration of the soft parts of a scorpion preserved in weak spirit and the cleaning of the chitinised ingrown cuticle give rise to the false appearance of a limb axis carrying the lamellæ. The

FIG. 19.

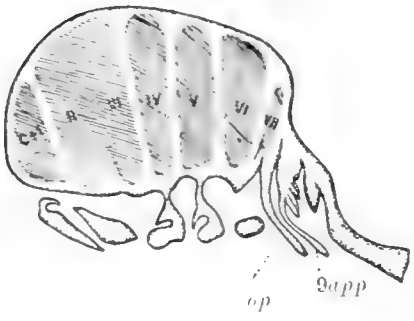


FIG. 20.

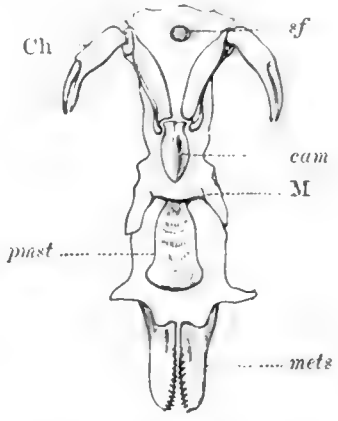


FIG. 19.—Section through an early embryo of Limulus longispina, showing seven transverse divisions in the region of the unsegmented anterior carapace. The seventh, VII, is anterior to the genital operculum, *op*, and is the cavity of the prægenital somite, which is more or less completely suppressed in subsequent development, possibly indicated by the great entopophyses of the prosomatic carapace. (After Kishinouye, 'Jour. Sci. Coll. Japan,' vol. v, 1892.)

FIG 20.—View of the ventral surface of the mid-line of the prosomatic region of Limulus polyphemus. The coxæ of the five pairs of limbs following the cheliceræ were arranged in a series on each side between the mouth, M, and the metasternites, *mets*. *sf*, the subfrontal median sclerite; Ch, the cheliceræ; *cam*, the camerostome or upper lip; M, the mouth; *pmst*, the promesosternal sclerite or chitinous plate, unpaired; *mets*, the right and left metasternites (corresponding to the similarly placed pentagonal sternite of Scorpio. Natural size. (After Lankester.)

margins of the lamellæ of the scorpion's lung-book which are lowermost in the figures (Fig. 15) and appear to be free are really those which are attached to the blood-holding axis. The true free ends are those nearest the stigma.

Passing on now from the mesosoma we come in Scorpio to the metasoma of six segments, the first of which is broad,

whilst the rest are cylindrical. The last is perforated by the anus, and carries the post-anal spine or sting. The somites of the metasoma carry no parapodia. In Limulus the metasoma is practically suppressed. In the allied extinct Eurypterines it is well developed, and resembles that of Scorpio. In the embryo Limulus (Fig. 42) the six somites of the mesosoma are not fused to form a carapace at an early stage, and they are followed by three separately marked metasomatic somites; the other three somites of the metasoma have disappeared in Limulus, but are represented by the unsegmented præanal region. It is probable that we have in the meta-

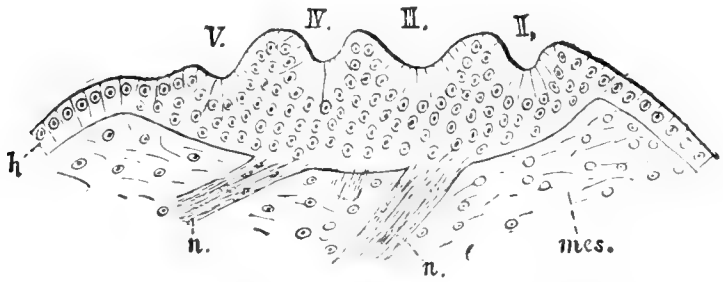


FIG. 21.—Development of the lateral eyes of a Scorpion. *h*, epidermic cell-layer; *mes*, mesoblastic connective tissue; *n*, nerves; II, III, IV, V, depressions of the epidermis in each of which a cuticular lens will be formed. (From Korschelt and Heider, after Laurie.)

soma of Limulus a case of the disappearance of once clearly demarcated somites. It would be possible to suppose, on the other hand, that new somites are only beginning to make their appearance here. The balance of various considerations is against the latter hypothesis. Following the metasoma in Limulus, we have as in Scorpio the post-anal spine—in this case not a sting, but a powerful and important organ of locomotion, serving to turn the animal over when it has fallen upon its back. The nature of the post-anal spine has been strangely misinterpreted by some writers. Owen (7) maintained that it represented a number of coalesced somites, regardless of its post-anal position and mode of development! The agreement of the grouping of the somites, of the form of

the parapodia (appendages, limbs) in each region, of the position of the genital aperture and operculum, of the position and character of the eyes, and of the powerful post-anal spines not seen in other Arthropods, is very convincing as to the affinity of Limulus and Scorpio. Perhaps the most important general agreement of Scorpio compared with Limulus and the Eurypterines is the division of the body into the three regions (or tagmata)—prosoma, mesosoma, and metasoma,—each consisting of six segments, the prosoma having leg-like appen-

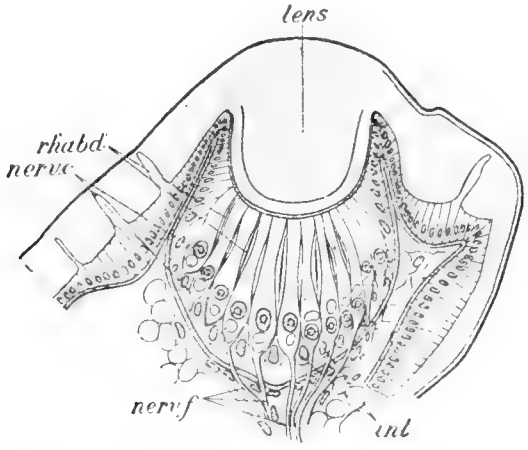


FIG. 22.—Section through the lateral eye of Euscorpius italicus. *lens*, cuticular lens; *nerv.c*, retinal cells (nerve-end cells); *rhabd*, rhabdomes; *nerv.f*, nerve-fibres of the optic nerve; *int*, intermediate cells (lying between the bases of the retinal cells). (After Lankester and Bourne, from Parker and Haswell's 'Text-book of Zoology,' Macmillan and Co.)

dages, the mesosoma having foliaceous appendages, and the metasoma being destitute of appendages.

In 1893, some years after the identification of the somites of Limulus with those of Scorpio, thus indicated, had been published, zoologists were startled by the discovery by a Japanese zoologist, Mr. Kishinouye (**8**), of a seventh prosomatic somite in the embryo of Limulus longispina. This was seen in longitudinal sections, as shown in Fig. 19. The simple identification of somite with somite in Limulus and Scorpio seemed to be threatened by this discovery. But in 1896 Dr. August Brauer, of Marburg (**9**), discovered in the

embryo of Scorpio a seventh prosomatic somite (see VIIPrG, Figs. 17 and 18), or, if we please so to term it, a prægenital somite, hitherto unrecognised. In the case of Scorpio this segment is indicated in the embryo by the presence of a pair of rudimentary appendages, carried by a well-marked somite. As in Limulus, so in Scorpio, this unexpected somite and its

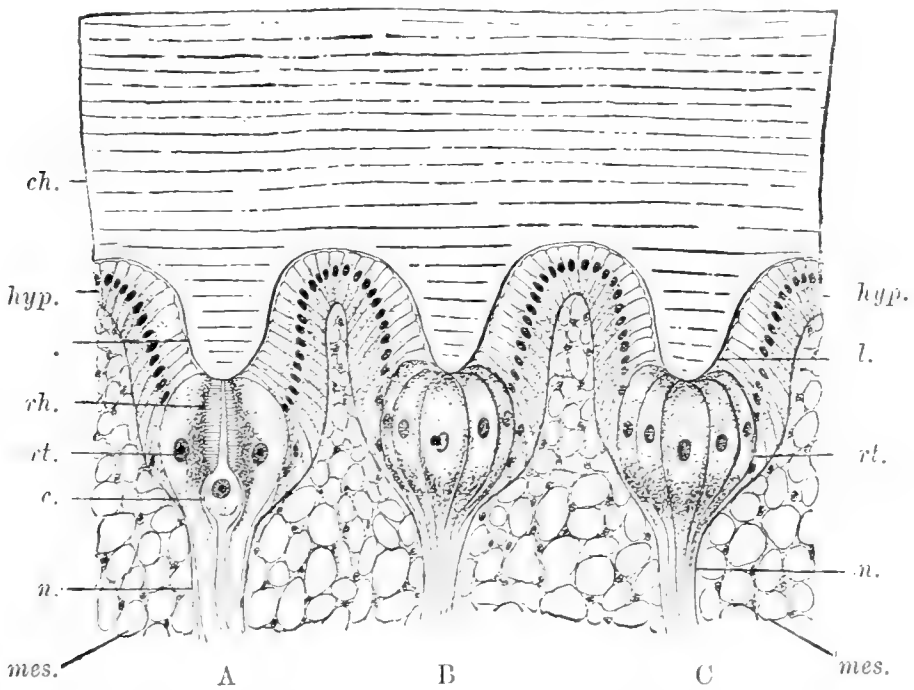


FIG. 23.—Section through a portion of the lateral eye of Limulus, showing three ommatidia, A, B, and C. *hyp*, the epidermic cell-layer (so-called hypodermis), the cells of which increase in volume below each lens, *l*, and become nerve-end cells or retinula cells, *rt*; in A the letters *rh* point to a rhabdomere secreted by the cell *rt*; *c*, the peculiar central spherical cell; *n*, nerve-fibres; *mes*, mesoblastic skeletal tissue; *ch*, chitinous cuticle. (From Korschelt and Heider, after Watase.)

appendages disappear in the course of development. In fact, more or less complete "excalation" of the somite takes place. Owing to its position it is convenient to term the somite which is excalated in Limulus and Scorpio "the prægenital somite." It appears not improbable that the sternal plates wedged in between the last pair of legs in both Scorpio and Limulus, viz. the pentagonal sternite of Scorpio (Fig. 10) and the

chilaria of Limulus (see Figs. 13 and 20), may in part represent in the adult the sternum of the excalated prægenital somite. This has not been demonstrated by an actual following out of the development, but the position of these pieces, and the fact that they are (in Limulus) supplied by an independent

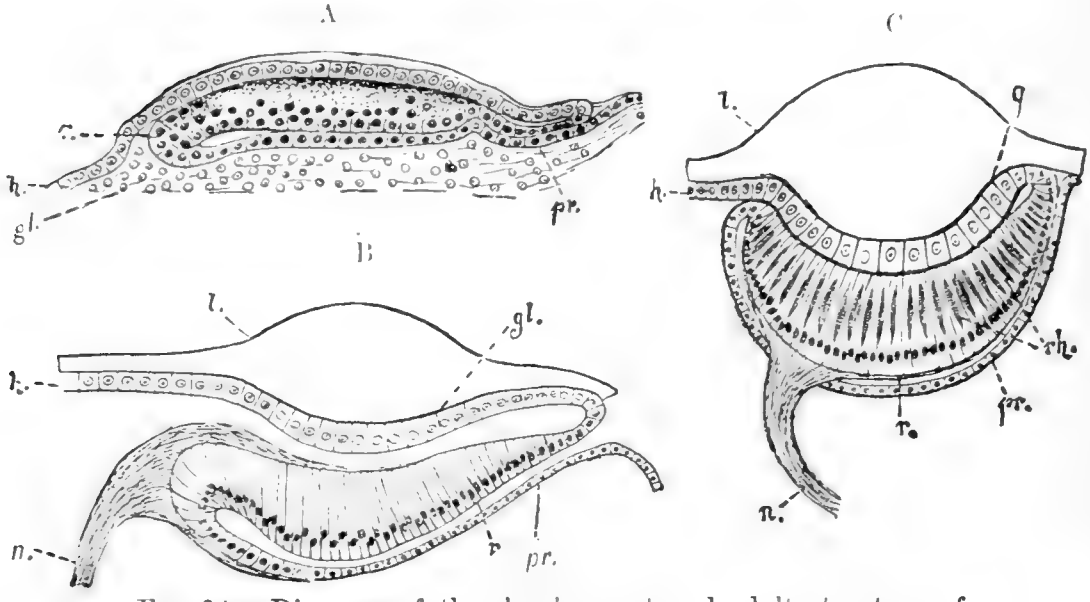


FIG. 24.—Diagrams of the development and adult structure of one of the paired central eyes of a Scorpion. A, early condition before the lens is deposited, showing the folding of the epidermic cell-layer into three; B, diagram showing the nature of this infolding; C, section through the fully formed eye; *h*, epidermic cell-layer; *r*, the retinal portion of the same which, owing to the infolding, lies between *gl*, the corneagen or lens-forming portion, and *pr*, the post-retinal or capsular portion or fold; *l*, cuticular lens; *g*, line separating lens from the lens-forming or corneagen cells of the epidermis; *n*, nerve-fibres; *rh*, rhabdomeres. (From Korschelt and Heider.) How the inversion of the nerve-end cells and their connection with the nerve-fibres is to be reconciled with the condition found in the adult, or with that of the monostichous eye, has not hitherto been explained.

segmental nerve, favours the view that they may comprise the sternal area of the vanished prægenital somite. This interpretation, however, of the "metasternites" of Limulus and Scorpio is opposed by the co-existence in Thelyphonus (Figs. 55, 57, and 58) of a similar metasternite with a complete prægenital somite. Hansen (**10**) has recognised that the

"prægenital somite" persists in a rudimentary condition, forming a "waist" to the series of somites in the Pedipalpi and Araneæ. The present writer is of opinion that it will be

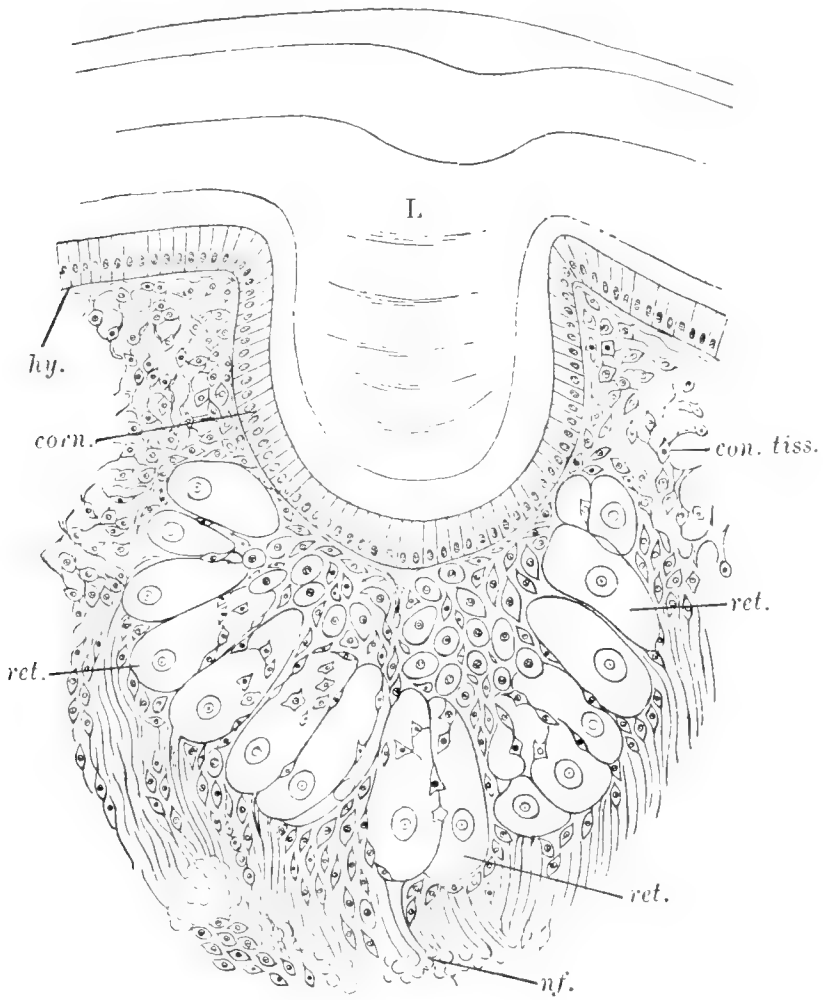


Fig. 25.—Section through one of the central eyes of a young Limulus. L, cuticular or corneous lens; *hy*, epidermic cell-layer; *corn.*, its corneagen portion immediately underlying the lens; *ret.*, retinula cells; *nf*, nerve-fibres; *con. tiss.*, connective tissue (mesoblastic skeletal tissue). (After Lankester and Bourne, 'Q. J. Micr. Sci.,' 1883.)

found most convenient to treat this evanescent somite as something special, and not to attempt to reckon it to either the prosoma or the mesosoma. These will then remain as typically composed each of six appendage-bearing somites—the prosoma

comprising in addition the ocular prosthomere.[1] When the prægenital somite or traces of it are present it should not be called "the seventh prosomatic" or the "first mesosomatic," but simply the "prægenital somite." The first segment of the mesosoma of Scorpio and Limulus thus remains the first segment, and can be identified as such throughout the Euarachnida, carrying as it always does the genital apertures. But it is necessary to remember, in the light of recent discoveries, that the sixth prosomatic pair of appendages is carried on the seventh somite of the whole series, there being two prosthomeres or somites in front of the mouth, the first carrying the eyes, the second the cheliceræ; also that the first mesosomatic or genital somite is not the seventh or even the eighth of the whole series of somites which have been historically present, but is the ninth, owing to the presence or to the excalation of a prægenital somite. It seems that confusion and trouble will be best avoided by abstaining from the introduction of the non-evident somites, the ocular and the prægenital, into the numerical nomenclature of the component somites of the three great body regions. We shall therefore, ignoring the ocular somite, speak of the first, second, third, fourth, fifth, and sixth leg-bearing somites of the prosoma, and indicate the appendages by the Roman numerals, I, II, III, IV, V, VI, and whilst ignoring the prægenital somite we shall speak of the first, second, third, etc., somite of the mesosoma or opisthosoma (united mesosoma and metasoma), and indicate them by the Arabic numerals.

There are a number of other important points of structure besides those referring to the somites and appendages in which Limulus agrees with Scorpio or other Arachnida, and differs from other Arthropoda. The chief of these are as follows:

1. The Composition of the Head (that is to say, of the anterior part of the prosoma), with especial reference to the Region in Front of the Mouth.—It appears (see

[1] See the article ARTHROPODA for the use of the term "prosthomere."

Arthropoda) that there is embryological evidence of the existence of two somites in Arachnida which were originally post-oral, but have become præoral by adaptational shifting of the oral aperture. These forwardly slipped somites are called "prosthomeres." The first of these has, in Arachnids

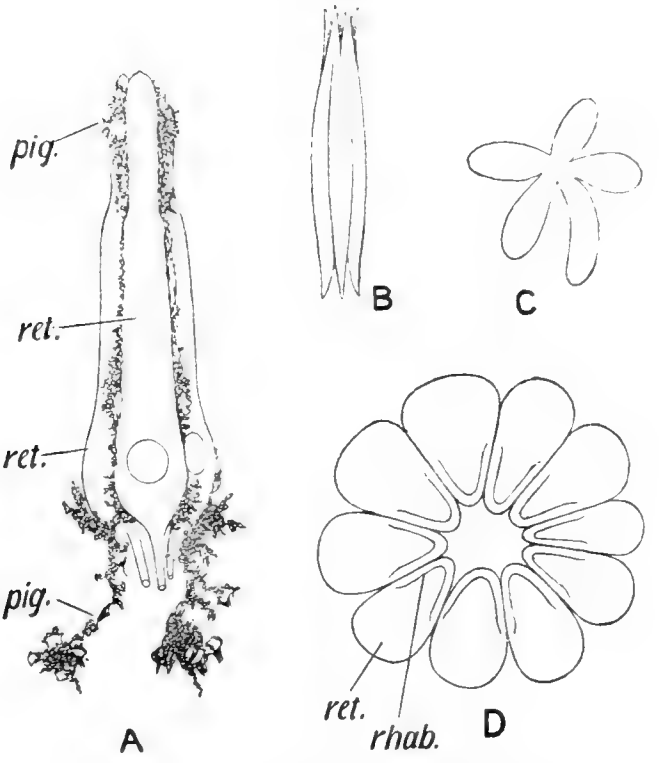


Fig. 26.—A, diagram of a retinula of the central eye of a Scorpion consisting of five retina cells (*ret.*), with adherent branched pigment cells (*pig.*); B, rhabdom of the same, consisting of five confluent rhabdomeres; C, transverse section of the rhabdom of a retinula of the Scorpion's central eye, showing its five constituent rhabdomeres as rays of a star; D, transverse section of a retinula of the lateral eye of Limulus, showing ten retinula cells, *ret.*, each bearing a rhabdomere, *rhab.* (After Lankester.)

as in other Arthropods, its pair of appendages represented by the eyes. The second has for its pair of appendages the small pair of limbs which in all living Arachnids is either chelate or retrovert (as in spiders), and is known as the cheliceræ. It is possible, as maintained by some writers (Patten and others), that the lobes of the cerebral nervous mass in Arachnids indicate a larger number of prosthomeres as having

fused in this region, but there is no embryological evidence at present which justifies us in assuming the existence in Arachnids of more than two prosthomeres. The position of the cheliceræ of Limulus, and of the ganglionic nerve-masses from which they receive their nerve-supply, is closely similar to that of the same structures in Scorpio. The cerebral mass is in Limulus more easily separated by dissection as a median lobe distinct from the laterally placed ganglia of the cheliceral somite than is the case in Scorpio, but the relations are practically the same in the two forms. Formerly it was supposed that in Limulus both the cheliceræ and the next following pair of appendages were prosthomerous, as in Crustacea; but the dissections of Alphonse Milne-Edwards (**6**) demonstrated the true limitations of the cerebrum, whilst embryological researches have done as much for Scorpio. Limulus thus agrees with Scorpio and differs from the Crustacea, in which there are three prosthomeres—one ocular and two carrying palpiform appendages. It is true that in the lower Crustacea (Apus, etc.) we have evidence of the gradual movement forward of the nerve-ganglia belonging to these palpiform appendages. But although in such lower Crustacea the nerve-ganglia of the third prosthomere have not fused with the anterior nerve-mass, there is no question as to the præoral position of the two appendage-bearing somites in addition to the ocular prosthomere. The Crustacea have, in fact, three prosthomeres in the head and the Arachnida only two, and Limulus agrees with the Arachnida in this respect, and differs from the Crustacea. The central nervous systems of Limulus and of Scorpio present closer agreement in structure than can be found when a crustacean is compared with either. The wide divarication of the lateral cords in the prosoma and their connection by transverse commissures, together with the "attraction" of ganglia to the prosomatic ganglion group which properly belong to hinder segments, are very nearly identical in the two animals. The form and disposition of the ganglion cells are also peculiar and closely similar in the two. (See Patten [**42**] for import-

ant observations on the neuromeres, etc., of Limulus and Scorpio.)

2. The Minute Structure of the Central Eyes and of the Lateral Eyes.—Limulus agrees with Scorpio not only in having a pair of central eyes and also lateral eyes, but in the microscopic structure of those organs, which differs in the central and lateral eyes respectively. The central eyes are "simple eyes,"—that is to say, have a single lens, and are hence called "monomeniscous." The lateral eyes are in Limulus "compound eyes,"—that is to say, consist of many lenses placed close together; beneath each lens is a complex of protoplasmic cells, in which the optic nerve terminates. Each such unit is termed an "ommatidium." The lateral eyes of Scorpio consist of groups of separate small lenses, each with its ommatidium, but they do not form a continuous compound eye as in Limulus. The ommatidium (soft structure beneath the lens-unit of a compound eye) is very simple in both Scorpio and Limulus. It consists of a single layer of cells, continuous with those which secrete the general chitinous covering of the prosoma. The cells of the ommatidium are a good deal larger than the neighbouring common cells of the epidermis. They secrete the knob-like lens (Fig. 22); but they also receive the nerve-fibres of the optic nerve. They are at the same time both optic nerve-end cells, that is to say, retina cells, and corneagen cells, or secretors of the chitinous lens-like cornea. In Limulus (Fig. 23) each ommatidium has a peculiar ganglion cell developed in a central position, whilst the ommatidium of the lateral eyelets of Scorpio shows small intermediate cells between the larger nerve-end cells. The structure of the lateral eye of Limulus was first described by Grenacher, and further and more accurately by Lankester and Bourne (5), and by Watase; that of Scorpio by Lankester and Bourne, who showed that the statements of von Graber were erroneous, and that the lateral eyes of Scorpio have a single-cell-layered or "monostichous" ommatidium like that of Limulus. Watase has shown in a very convincing way how, by deepening the pit-

like set of cells beneath a simple lens, the more complex ommatidia of the compound eyes of Crustacea and Hexapoda may be derived from such a condition as that presented in the lateral eyes of Limulus and Scorpio. (For details the reader is referred to Watase [11], and to Lankester and Bourne [5].) The structure of the central eyes of Scorpio and spiders, and also of Limulus, differs essentially from that of the lateral eyes in having two layers of cells (hence called diplostichous) beneath the lens, separated from one another by a membrane (Figs. 24 and 25). The upper layer is the corneagen, and secretes the lens; the lower is the retinal layer. The mass of soft cell-structures beneath a large lens of a central eye is called an "ommatœum." It shows in Scorpio and Limulus a tendency to segregate into minor groups or "ommatidia." It is found that in embryological growth the retinal layer of the central eyes forms as a separate pouch, which is pushed in laterally beneath the corneagen layer from the epidermic cell layer. Hence it is in origin double, and consists of a true retinal layer and a post-retinal layer (Fig. 24, B), though these are not separated by a membrane. Accordingly the diplostichous ommatœum or soft tissue of the Arachnid's central eye should strictly be called "triplostichous," since the deep layer is itself doubled or folded. The retinal cells of both the lateral and central eyes of Limulus and Scorpio produce cuticular structures on their sides; each such piece is a rhabdomere, and a number (five or ten) uniting form a rhabdom (Fig. 26). In the specialised ommatidia of the compound eyes of Crustacea and Hexapods the rhabdom is an important structure.[1] It is a very significant fact that the lateral and central eyes of Limulus and Scorpio not only agree each with each in regard to their monostichous and diplostichous structure, but also in the formation in both classes of eyes of rhabdomeres and rhabdoms in which the component pieces are five or a multiple of five (Fig. 26). Whilst each unit of the lateral eye of Limulus has a rhabdom

[1] See Fig. 11 in the article ARTHROPODA.

of ten[1] pieces forming a star-like chitinous centre in section, each lateral eye of Scorpio has several rhabdoms of five or less rhabdomeres, indicating that the Limulus lateral eye-unit is more specialised than the detached lateral eyelet of Scorpio, so as to present a coincidence of one lens with one rhabdom. Numerous rhabdomeres (grouped as rhabdoms in Limulus) are found in the retinal layer of the central eyes also.

Whilst Limulus agrees thus closely with Scorpio in regard to the eyes, it is to be noted that no Crustacean has structures corresponding to the peculiar diplostichous central eyes, though these occur again (with differences in detail) in Hexapoda. Possibly, however, an investigation of the development of the median eyes of some Crustacea (Apus, Palæmon) may prove them to be diplostichous in origin.

3. The So-called "Coxal Glands."—In 1882 ('Proc. Roy. Soc.,' No. 221) Lankester described under the name "coxal glands" a pair of brilliantly white oviform bodies lying in the scorpion's prosoma immediately above the coxæ of the fifth and sixth pairs of legs (Fig. 27). These bodies had been erroneously supposed by Newport (12) and other observers to be glandular outgrowths of the alimentary canal. They are really excretory glands, and communicate with the exterior by a very minute aperture on the posterior face of the coxa of the fifth limb on each side. When examined with the microscope, by means of the usual section method, they are seen to consist of a labyrinthine tube lined with peculiar cells, each cell having a deep vertically striated border on the surface farthest from the lumen, as is seen in the cells of some renal organs. The coils and branches of the tube are packed by connective tissue and blood-spaces. A similar pair of coxal glands, lobate instead of ovoid in shape, was described by Lankester in Mygale, and it was also shown by him that the structures in Limulus called

[1] Though ten is the prevailing number of retinula cells and rhabdomeres in the lateral eye of Limulus, Watase states that they may be as few as nine and as many as eighteen.

"brick-red glands" by Packard have the same structure and position as the coxal glands of Scorpio and Mygale. In Limulus these organs consist each of four horizontal lobes lying on the coxal margin of the second, third, fourth, and fifth prosomatic limbs, the four lobes being connected to one another by a transverse piece or stem (Fig. 28). Micro-

Fig. 27.

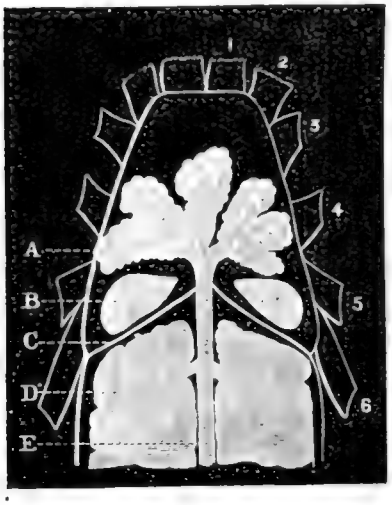


Fig. 28.

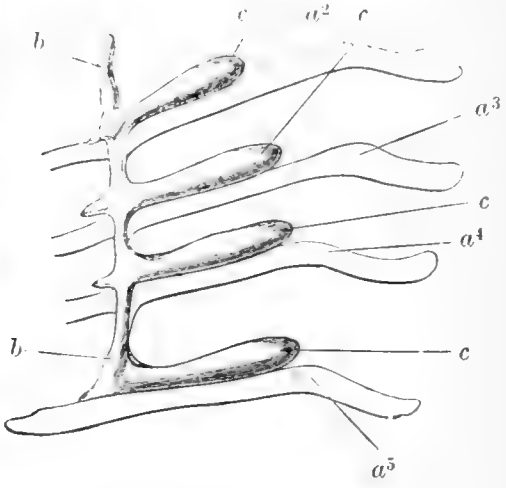


Fig. 27.—Diagram showing the position of the coxal glands of a Scorpion, Buthus australis, Lin., in relation to the legs, diaphragm (entosternal flap), and the gastric cæca. 1 to 6, the bases of the six prosomatic limbs; A, prosomatic gastric gland (sometimes called salivary); B, coxal gland; C, diaphragm of Newport = fibrous flap of the entosternum; D, mesosomatic gastric cæca (so-called liver); E, alimentary canal. (From Lankester, 'Q. J. Micr. Sci.,' vol. xxiv, N.S., p. 152.)

Fig. 28.—The right coxal gland of Limulus polyphemus, Latr. a^2 to a^5, posterior borders of the chitinous bases of the coxæ of the second, third, fourth, and fifth prosomatic limbs; *b*, longitudinal lobe or stolon of the coxal gland; *c*, its four transverse lobes or outgrowths corresponding to the four coxæ. (From Lankester, loc. cit., after Packard.)

scopically their structure is the same in essentials as that of the coxal glands of Scorpio (**13**). Coxal glands have since been recognised and described in other Arachnida. It has lately (1900) been shown that the coxal gland of Limulus is provided with a very delicate thin-walled coiled duct which

opens, even in the adult condition, by a minute pore on the coxa of the fifth leg (Patten and Hazen [**13A**]). Previously to this, Lankester's pupil Gulland had shown (1885) that in the embryo the coxal gland is a comparatively simple tube, which opens to the exterior in this position, and by its other extremity into a cœlomic space. Similar observations were made by Laurie (**17**) in Lankester's laboratory (1890) with regard to the early condition of the coxal gland of Scorpio, and by Bertkau (**41**) as to that of the spider Atypus. H. M. Bernard (**13B**) showed that the opening remains in the adult scorpion. In all the embryonic or permanent opening is on the coxa of the fifth pair of prosomatic limbs. Thus an organ newly discovered in Scorpio was found to have its counterpart in Limulus.

The name "coxal gland" needs to be carefully distinguished from "crural gland," with which it is apt to be confused. The crural glands, which occur in many terrestrial Arthropods, are epidermal in origin and totally distinct from the coxal glands. The coxal glands of the Arachnida are structures of the same nature as the green glands of the higher Crustacea and the so-called "shell glands" of the Entomostraca. The latter open at the base of the fifth pair of limbs of the Crustacean, just as the coxal glands open on the coxal joint of the fifth pair of limbs of the Arachnid. Both belong to the category of "cœlomoducts," namely, tubular or funnel-like portions of the cœlom opening to the exterior in pairs in each somite (potentially), and usually persisting in only a few somites as either "urocœls" (renal organs) or "gonocœls" (genital tubes). In Peripatus they occur in every somite of the body. They have till recently been very generally identified with the nephridia of Chætopod worms, but there is good reason for considering the true nephridia (typified by the nephridia of the earthworm) as a distinct class of organs (see Lankester in vol. ii, chap. iii, of 'A Treatise on Zoology,' 1900). The genital ducts of Arthropoda are like the green glands, shell glands, and coxal glands, to be regarded as cœlomoducts (gonocœls).

The coxal glands do not establish any special connection between Limulus and Scorpio, since they also occur in the same somite in the lower Crustacea, but it is to be noted that the coxal glands of Limulus are in minute structure and probably in function more like those of Arachnids than those of Crustacea.

4. The Entosternites and their Minute Structure. —Straus-Durkheim (1) was the first to insist on the affinity between Limulus and the Arachnids, indicated by the presence of a free suspended entosternum or plastron or entosternite in both. We have figured here (Figs. 1—6) the entosternites of Limulus, Scorpio, and Mygale. Lankester some years ago made a special study of the histology (3) of these entosternites for the purpose of comparison, and also ascertained the relations of the very numerous muscles which are inserted into them (4). The entosternites are cartilaginous in texture, but they have neither the chemical character nor the microscopic structure of the hyaline cartilage of Vertebrates. They yield chitin in place of chondrin or gelatine—as does also the cartilage of the Cephalopod's endoskeleton. In microscopic structure they all present the closest agreement with one another. We find a firm, homogeneous, or sparsely fibrillated matrix in which are embedded nucleated cells (corpuscles of protoplasm) arranged in rows of three, six, or eight parallel with the adjacent lines of fibrillation.

A minute entosternite having the above-described structure is found in the Crustacean Apus between the bases of the mandibles, and also in the Decapoda in a similar position, but in no Crustacean does it attain to any size or importance. On the other hand, the entosternite of the Arachnida is a very large and important feature in the structure of the prosoma, and must play an important part in the economy of these organisms. In Limulus (Figs. 1 and 2) it has as many as twenty-five pairs of muscles attached to it, coming to it from the bases of the surrounding limbs and from the dorsal carapace and from the pharynx. It consists of an oblong

plate two inches in length and one in breadth, with a pair of tendinous outgrowths standing out from it at right angles on each side. It "floats" between the prosomatic nerve centres and the alimentary canal. In each somite of the mesosoma is a small, free entosternite having a similar position, but below or ventrad of the nerve-cords, and having a smaller number of muscles attached to it. The entosternite was probably in origin part of the fibrous connective tissue lying close to the integument of the sternal surface—giving attachment to muscles corresponding more or less to those at present attached to it. It became isolated and detached, why or with what advantage to the organism it is difficult to say, and at that period of Arachnidan development the great ventral nerve-cords occupied a more lateral position than they do at present. We know that such a lateral position of the nerve-cords preceded the median position in both Arthropoda and Chætopoda. Subsequently to the floating off of the entosternite the approximation of the nerve-cords took place in the prosoma, and thus they were able to take up a position below the entosternite. In the mesosoma the approximation had occurred before the entosternites were formed.

In the scorpion (Figs. 3 and 4) the entosternite has tough membrane-like outgrowths which connect it with the body-wall, both dorsally and ventrally forming an oblique diaphragm, cutting off the cavity of the prosoma from that of the mesosoma. It was described by Newport as "the diaphragm." Only the central and horizontal parts of this structure correspond precisely to the entosternite of Limulus: the right and left anterior processes (marked *ap* in Figs. 3 and 4, and RAP, LAP, in Figs. 1 and 2) correspond in the two animals, and the median lateral process *lmp* of the scorpion represents the tendinous outgrowths ALR, PLR of Limulus. The scorpion's entosternite gives rise to outgrowths, besides the great posterior flaps, *pf*, which form the diaphragm, unrepresented in Limulus. These are a ventral arch forming a neural canal through which the great nerve-

cords pass (Figs. 3 and 4, *snp*), and further a dorsal gastric canal and arterial canal which transmit the alimentary tract and the dorsal artery respectively (Figs. 3 and 4, GC, DR).

In Limulus small entosternites are found in each somite of the appendage-bearing mesosoma, and we find in Scorpio, in the only somite of the mesosoma which has a well-developed pair of appendages, that of the pectens, a small entosternite with ten pairs of muscles inserted into it. The supra-pectinal entosternite lies ventrad of the nerve-cords.

In Mygale (Figs. 5 and 6) the form of the entosternite is more like that of Limulus than is that of Scorpio. The anterior notch Ph.N. is similar to that in Limulus, and the pairs of upstanding tendons correspond to the similar pairs in Limulus, whilst the imbricate triangular pieces of the posterior median region resemble the similarly placed structures of Limulus in a striking manner.

It must be confessed that we are singularly ignorant as to the functional significance of these remarkable organs—the entosternites. Their movement in an upward or downward direction in Limulus and Mygale must exert a pumping action on the blood contained in the dorsal arteries and the ventral veins respectively. In Scorpio the completion of the horizontal plate by oblique flaps, so as to form an actual diaphragm shutting off the cavity of the prosoma from the rest of the body, possibly gives to the organs contained in the anterior chamber a physiological advantage in respect of the supply of arterial blood and its separation from the venous blood of the mesosoma. Possibly the movement of the diaphragm may determine the passage of air into or out of the lung-sacs. Muscular fibres connected with the suctorial pharynx are in Limulus inserted into the entosternite, and the activity of the two organs may be correlated.

5. The Blood and the Blood-vascular System.—The blood fluids of Limulus and Scorpio are very similar. Not only are the blood-corpuscles of Limulus more like in form and granulation to those of Scorpio than to those of

any Crustacean, but the fluid is in both animals strongly impregnated with the blue-coloured respiratory proteid hæmocyanin. This body occurs also in the blood of Crustacea and of Molluscs, but its abundance in both Limulus and Scorpio is very marked, and gives to the freshly shed blood a strong indigo-blue tint.

The great dorsal contractile vessel or "heart" of Limulus is closely similar to that of Scorpio; its ostia or incurrent orifices are placed in the same somites as those of Scorpio, but there is one additional posterior pair. The origin of the paired arteries from the heart differs in Limulus from the arrangement obtaining in Scorpio, in that a pair of lateral commissural arteries exist in Limulus (as described by Alphonse Milne-Edwards [**6**]) leading to a suppression of the more primitive direct connection of the four pairs of posterior lateral arteries, and of the great median posterior arteries with the heart itself (Fig. 29). The arterial system is very completely developed in both Limulus and Scorpio, branching repeatedly until minute arterioles are formed, not to be distinguished from true capillaries; these open into irregular swollen vessels which are the veins or venous sinuses. A very remarkable feature in Limulus, first described by Owen, is the close accompaniment of the prosomatic nerve centres and nerves by arteries, so close indeed that the great ganglion mass and its outrunning nerves are actually sunk in or invested by arteries. The connection is not so intimate in Scorpio, but is nevertheless a very close one, closer than we find in any other Arthropods in which the arterial system is well developed, e. g. the Myriapoda and some of the arthrostracous Crustacea. It seems that there is a primitive tendency in the Arthropoda for the arteries to accompany the nerve-cords, and a "supra-spinal" artery—that is to say, an artery in close relation to the ventral nerve-cords—has been described in several cases. On the other hand, in many Arthropods, especially those which possess tracheæ, the arteries do not have a long course, but soon open into wide blood-sinuses. Scorpio certainly

Fig. 29. Fig. 30.

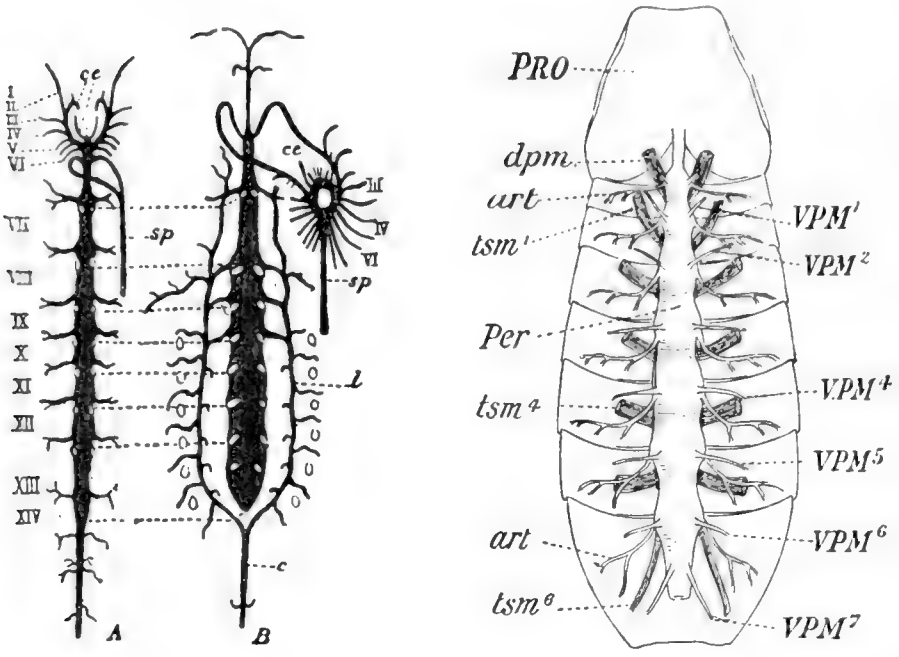


Fig. 29.—Diagram of the arterial system of A, Scorpio, and B, Limulus. The Roman numerals indicate the body somites and the two figures are adjusted for comparison. *ce*, cerebral arteries; *sp*, supra-spinal or medullary artery; *c*, caudal artery; *l*, lateral anastomotic artery of Limulus. The Roman numerals indicate the body somites, and the two figures are adjusted for comparison. *ce*, cerebral arteries; *sp*, supra-spinal or medullary artery; *c*, caudal artery; *l*, lateral anastomotic artery of Limulus. The figure B also shows the peculiar neural investiture formed by the cerebral arteries in Limulus and the derivation from this of the arteries to the limbs, III, IV, VI, whereas in Scorpio the latter have a separate origin from the anterior aorta. (From Lankester, "Limulus an Arachnid.")

Fig. 30.—View from below of a Scorpion (B. occitanus) opened and dissected so as to show the pericardium with its muscles, the lateral arteries, and the tergo-sternal muscles. *PRO*, prosoma; *dpm*, dorso-plastral muscle; *art*, lateral artery; *tsm*^{1}, tergo-sternal muscle (labelled *dv* in Fig. 31) of the second (pectiniferous) mesosomatic somite: this is the most anterior pair of the series of six—none are present in the genital somite; *tsm*^{4}, tergo-sternal muscle of the fifth mesosomatic somite; *tsm*^{6}, tergo-sternal muscle of the enlarged first mesosomatic somite; *Per*, pericardium; *VPM*^{1} to *VPM*^{7}, the series of seven pairs of veno-pericardiac muscles (labelled *pv* in Fig. 31). There is some reason to admit the existence of another more anterior pair of these muscles in Scorpio; this would make the number exactly correspond with the number in Limulus. (After Lankester, 'Trans. Zool. Soc.,' vol. xi, 1883.)

comes nearer to Limulus in the high development of its arterial system, and the intimate relation of the anterior aorta and its branches to the nerve centres and great nerves, than does any other Arthropod.

An arrangement of great functional importance in regard to the venous system must now be described, which was shown in 1883 by Lankester to be common to Limulus and Scorpio. This arrangement has not hitherto been detected in any other class than the Arachnida, and if it should ultimately prove to be peculiar to that group, would have

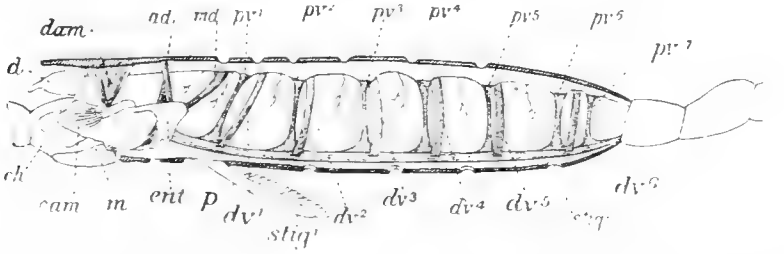


FIG. 31.—Diagram of a lateral view of a longitudinal section of a Scorpion. *d*, chelicera; *ch*, chela; *cam*, camerostome; *m*, mouth; *ent*, entosternum; *p*, pecten; *stig*¹, first pulmonary aperture; *stig*⁴, fourth pulmonary aperture; *dam*, muscle from carapace to a præoral entosclerite; *ad*, muscle from carapace to entosternum; *md*, muscle from tergite of genital somite to entosternum (same as *dpm* in Fig. 30); *dv*¹ to *dv*⁶, dorso-ventral muscles (same as the series labelled *tsm* in Fig. 30); *pv*¹ to *pv*⁷, the seven veno-pericardiac muscles of the right side (labelled *VPM* in Fig. 30). (After Beck, 'Trans. Zool. Soc.,' vol. xi, 1883.)

considerable weight as a proof of the close genetic affinity of Limulus and Scorpio.

The great pericardial sinus is strongly developed in both animals. Its walls are fibrous and complete, and it holds a considerable volume of blood when the heart itself is contracted. Opening in pairs in each somite, right and left into the pericardial sinus are large veins, which bring the blood respectively from the gill-books and the lung-books to that chamber, whence it passes by the ostia into the heart. The blood is brought to the respiratory organs in both cases by a great venous-collecting sinus having a ventral median position. In both animals the wall of the pericardial

sinus is connected by vertical muscular bands to the wall of the ventral venous sinus (its lateral expansions around the lung-books in Scorpio) in each somite through which the pericardium passes. There are seven pairs of these veno-pericardiac vertical muscles in Scorpio, and eight in Limulus (see Figs. 30—32). It is obvious that the contraction of these muscles must cause a depression of the floor of the pericardium and a rising of the roof of the ventral blood-sinus, and a consequent increase of

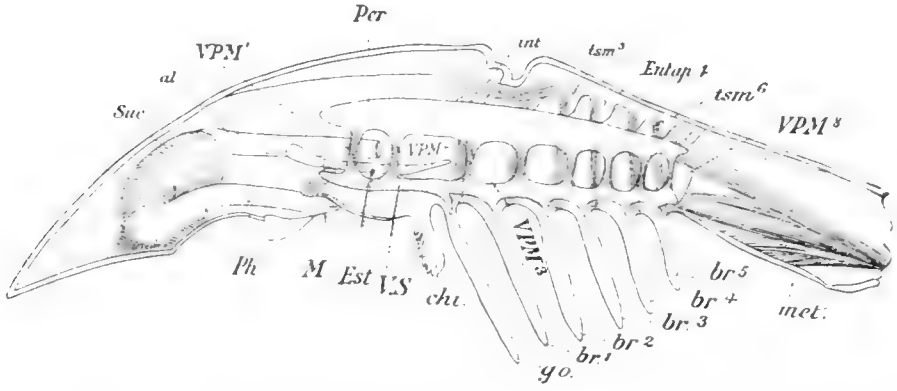


FIG. 32.—Diagram of a lateral view of a longitudinal section of Limulus. *Suc*, suctorial pharynx; *al*, alimentary canal; *Ph*, pharynx; *M*, mouth; *Est*, entosternum; *VS*, ventral venous sinus; *chi*, chilaria; *go*, genital operculum; br^1 to br^5, branchial appendages; *met*, unsegmented metasoma; $entap^4$, fourth dorsal entapophysis of left side; *tsm*, tergo-sternal muscles, six pairs as in Scorpio (labelled *dv* in Fig. 31); VPM^1 to VPM^8, the eight pairs of veno-pericardiac muscles (labelled *pv* in Fig. 31). VPM^1 is probably represented in Scorpio, though not marked in Figs. 30 and 31. (After Benham, 'Trans. Zool. Soc.,' vol. xi, 1883.)

volume and flow of blood to each. Whether the pericardium and the ventral sinus are made to expand simultaneously or all the movement is made by one only of the surfaces concerned must depend on conditions of tension. In any case it is clear that we have in these muscles an apparatus for causing the blood to flow differentially in increased volume into either the pericardium, through the veins leading from the respiratory organs, or from the body generally into the great sinuses which bring the blood to the respiratory

organs. These muscles act so as to pump the blood through the respiratory organs.

It is not surprising that with so highly developed an arterial system Limulus and Scorpio should have a highly developed mechanism for determining the flow of blood to the respiratory organs. That this is, so to speak, a need of animals with localised respiratory organs is seen by the existence of provisions serving a similar purpose in other animals, e. g. the branchial hearts of the Cephalopoda.

The veno-pericardiac muscles of Scorpio were seen and figured by Newport but not described by him. Those of Limulus were described and figured by Alphonse Milne-Edwards, but he called them merely "transparent ligaments," and did not discover their muscular structure. They are figured and their importance for the first time recognised in the memoir on the muscular and skeletal systems of Limulus and Scorpio by Lankester, Beck, and Bourne (4).

6. Alimentary Canal and Gastric Glands.—The alimentary canal in Scorpio, as in Limulus, is provided with a powerful suctorial pharynx, in the working of which extrinsic muscles take a part. The mouth is relatively smaller in Scorpio than in Limulus—in fact, is minute, as it is in all the terrestrial Arachnida which suck the juices of either animals or plants. In both the alimentary canal takes a straight course from the pharynx (which bends under it downwards and backwards towards the mouth in Limulus) to the anus, and is a simple, narrow, cylindrical tube (Fig. 33). The only point in which the gut of Limulus resembles that of Scorpio rather than that of any of the Crustacea is in possessing more than a single pair of ducts or lateral outgrowths connected with ramified gastric glands or gastric cæca. Limulus has two pairs of these, Scorpio as many as six pairs. The Crustacea never have more than one pair. The minute microscopic structure of the gastric glands in the two animals is practically identical. The functions of these gastric diverticula have never been carefully investigated.

It is very probable that in Scorpio they do not serve merely to secrete a digestive fluid (shown in other Arthropoda to resemble the pancreatic fluid), but that they also become

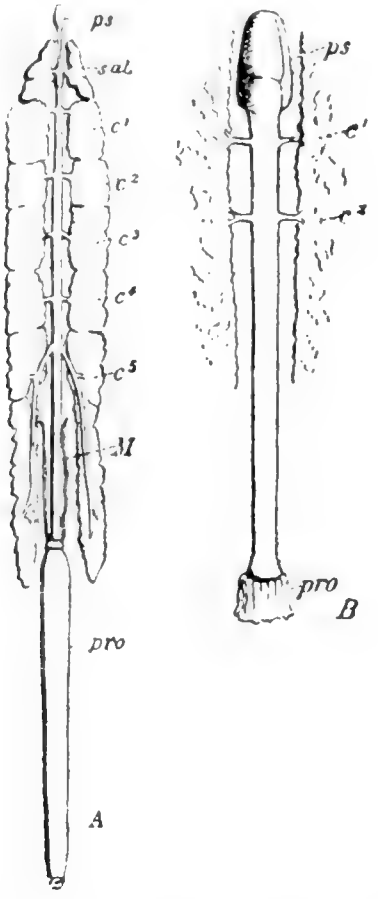


FIG. 33.—The alimentary canal and gastric glands of a Scorpion (A) and of Limulus (B). *ps*, muscular suctorial enlargement of the pharynx; *sal*, prosomatic pair of gastric cæca in Scorpio, called salivary glands by some writers; c^1 and c^2, the anterior two pairs of gastric cæca and ducts of the mesosomatic region; c^3, c^4, and c^5, cæca and ducts of Scorpio not represented in Limulus; *M*, the Malpighian or renal cæcal diverticula of Scorpio; *pro*, the proctodæum or portion of gut leading to anus, and formed embryologically by an inversion of the epiblast at that orifice. (From Lankester, "Limulus an Arachnid.")

distended by the juices of the prey sucked in by the scorpion —as certainly must occur in the case of the simple unbranched gastric cæca of the spiders.

The most important difference which exists between the

structure of Limulus and that of Scorpio is found in the hinder region of the alimentary canal. Scorpio is here provided with a single or double pair of renal excretory tubes, which have been identified by earlier authors with the Malpighian tubes of the Hexapod and Myriapod insects. Limulus is devoid of any such tubes. We shall revert to this subject below.

7. Ovaries and Spermaries; Gonocœls and Gonoducts.—The scorpion is remarkable for having the specialised portion of cœlom, from the walls of which egg-cells or sperm-cells are developed according to sex, in the form of a simple but extensive network. It is not a pair of simple tubes, nor of dendriform tubes, but a closed network. The same fact is true of Limulus, as was shown by Owen (**7**) in regard to the ovary, and by Benham (**14**) in regard to the testis. This is a very definite and remarkable agreement, since such a reticular gonocœl is not found in Crustacea (except in the male Apus). Moreover there is a significant agreement in the character of the spermatozoa of Limulus and Scorpio. The Crustacea are—with the exception of the Cirrhipedia—remarkable for having stiff, motionless spermatozoids. In Limulus Lankester found (**15**) the spermatozoa to possess active flagelliform "tails," and to resemble very closely those of Scorpio, which, as are those of most terrestrial Arthropoda, are actively motile. This is a microscopic point of agreement, but is none the less significant.

In regard to the important structures concerned with the fertilisation of the egg, Limulus and Scorpio differ entirely from one another. The eggs of Limulus are fertilised in the sea after they have been laid. Scorpio, being a terrestrial animal, fertilises by copulation. The male possesses elaborate copulatory structures of a chitinous nature, and the eggs are fertilised in the female without even quitting the place where they are formed on the wall of the reticular gonocœl. The female scorpion is viviparous, and the young are produced in a highly developed condition as fully formed scorpions.

Differences between Limulus and Scorpio.—We have now passed in review the principal structural features in which Limulus agrees with Scorpio and differs from other Arthropoda. There remains for consideration the one important structural difference between the two animals. Limulus agrees with the majority of the Crustacea in being destitute of renal excretory cæca or tubes opening into the hinder part of the gut. Scorpio, on the other hand, in common with all air-breathing Arthropoda except Peripatus, possesses these tubules, which are often called Malpighian tubes. A great deal has been made of this difference by some writers. It has been considered by them as proving that Limulus, in spite of all its special agreements with Scorpio (which, however, have scarcely been appreciated by the writers in question), really belongs to the Crustacean line of descent; whilst Scorpio, by possessing Malpighian tubes, is declared to be unmistakably tied together with the other Arachnida to the tracheate Arthropods, the Hexapods, Diplopods, and Chilopods, which all possess Malpighian tubes.

It must be pointed out that the presence or absence of such renal excretory tubes opening into the intestine appears to be a question of adaptation to the changed physiological conditions of respiration, and not of morphological significance, since a pair of renal excretory tubes of this nature is found in certain Amphipod Crustacea (Talorchestia, etc.) which have abandoned a purely aquatic life. This view has been accepted and supported by Professors Korschelt and Heider (**16**). An important fact in its favour was discovered by Laurie (**17**), who investigated the embryology of two species of Scorpio under Lankester's direction. It appears that the Malpighian tubes of Scorpio are developed from the mesenteron, viz. that portion of the gut which is formed by the hypoblast; whereas in Hexapod insects the similar cæcal tubes are developed from the proctodæum or inpushed portion of the gut, which is formed from epiblast. In fact, it is not possible to maintain that the renal excretory tubes

of the gut are of one common origin in the Arthropoda. They have appeared independently in connection with a change in the excretion of nitrogenous waste in Arachnids, Crustacea, and the other classes of Arthropoda when aërial, as opposed to aquatic respiration has been established—and they have been formed in some cases from the mesenteron, in other cases from the proctodæum. Their appearance in the air-breathing Arachnids does not separate those forms from the water-breathing Arachnids, which are devoid of them, any more than does their appearance in certain Amphipoda separate those Crustaceans from the other members of the class.

Further, it is pointed out by Korschelt and Heider that the hinder portion of the gut frequently acts in Arthropoda as an organ of nitrogenous excretion in the absence of any special excretory tubules, and that the production of such cæca from its surface in separate lines of descent does not involve any elaborate or unlikely process of growth. In other words, the Malpighian tubes of the terrestrial Arachnida are homoplastic with those of Hexapoda and Myriapoda, and not homogenetic with them. We are compelled to take a similar view of the agreement between the tracheal air-tubes of Arachnida and other tracheate Arthropods. They are homoplasts (see **18**) one of another, and do not owe their existence in the various classes compared to a common inheritance of an ancestral tracheal system.

Conclusions arising from the Close Affinity of Limulus and Scorpio.—When we consider the relationships of the various classes of Arthropoda, having accepted and established the fact of the close genetic affinity of Limulus and Scorpio, we are led to important conclusions. In such a consideration we have to make use not only of the fact just mentioned, but of three important generalisations, which serve, as it were, as implements for the proper estimation of the relationships of any series of organic forms. First of all there is the generalisation that the relationships of the various

forms of animals (or of plants) to one another is that of the ultimate twigs of a much-branching genealogical tree. Secondly, identity of structure in two organisms does not necessarily indicate that the identical structure has been inherited from an ancestor common to the two organisms compared (homogeny), but may be due to independent development of a like structure in two different lines of descent (homoplasy). Thirdly, those members of a group which, whilst exhibiting undoubted structural characters indicative of their proper assignment to that group, yet are simpler than and inferior in elaboration of their organisation to other members of the group, are not necessarily representatives of the earlier and primitive phases in the development of the group, but are very often examples of retrogressive change or degeneration. The second and third implements of analysis above cited are of the nature of cautions or checks. Agreements are not *necessarily* due to common inheritance; simplicity is not *necessarily* primitive and ancestral.

On the other hand, we must not rashly set down agreements as due to "homoplasy" or "convergence of development" if we find two or three or more concurrent agreements. The probability is against agreement being due to homoplasy when the agreement involves a number of really separate (not correlated) coincidences. Whilst the chances are in favour of some *one* homoplastic coincidence or structural agreement occurring between some member or other of a large group *a*, and some member or other of a large group *b*, the matter is very different when by such an initial coincidence the two members have been particularised. The chances against these two selected members exhibiting *another* really independent homoplastic agreement are enormous; let us say 10,000 to 1. The chances against yet another coincidence are a hundred million to one, and against yet one more "coincidence" they are the square of a hundred million to one. Homoplasy can only be assumed where the coincidence is of a simple nature, and is such as may be reasonably supposed to have arisen by the action of like selective

conditions upon like material in two separate lines of descent.[1]

So, too, degeneration is not to be lightly assumed as the explanation of a simplicity of structure. There is a very definite criterion of the simplicity due to degeneration, which can in most cases be applied. Degenerative simplicity is never uniformly distributed over all the structures of the organism. It affects many or nearly all the structures of the body, but leaves some—it may be only one—at a high level of elaboration and complexity. Ancestral simplicity is more uniform, and does not co-exist with specialisation and elaboration of a single organ. Further, degeneration cannot be inferred safely by the examination of an isolated case: usually we obtain a series of forms indicating the steps of a change in structure; and what we have to decide is whether the movement has been from the simple to the more complex, or from the more complex to the simple. The feathers of a peacock afford a convenient example of primitive and degenerative simplicity. The highest point of elaboration in colour, pattern, and form is shown by the great eye-painted tail feathers. From these we can pass by gradual transitions in two directions, viz. either to the simple lateral tail feathers, with a few rami only, developed only on one side of the shaft and of uniform metallic coloration—or to the simple contour feathers of small size, with the usual symmetrical series of numerous rami right and left of the shaft and no remarkable colouring. The one-sided specialisation and the peculiar metallic colouring of the lateral tail feathers mark them as the extreme terms of a degenerative series; whilst

[1] A great deal of superfluous hypothesis has lately been put forward in the name of "the principle of convergence of characters" by a certain school of palæontologists. The horse is supposed by these writers to have originated by separate lines of descent in the Old World and the New, from five-toed ancestors! And the important consequences following from the demonstration of the identity in structure of Limulus and Scorpio are evaded by arbitrary and even fantastic invocations of a mysterious transcendental force which brings about "convergence" irrespective of heredity and selection. Morphology becomes a farce when such assumptions are made.

the symmetry, likeness of constituent parts inter se, and absence of specialised pigment, as well as the fact that they differ little from any average feather of birds in general, mark the contour feather as primitively simple, and as the starting-point from which the highly elaborated eye-painted tail feather has gradually evolved.

Applying these principles to the consideration of the Arachnida, we arrive at the conclusion that the smaller and simpler Arachnids are not the more primitive, but that the Acari or mites are, in fact, a degenerate group. This was maintained by Lankester in 1878 (**19**), again in 1881 (**20**); it was subsequently announced as a novelty by Claus in 1885 (**21**). Though the aquatic members of a class of animals are in some instances derived from terrestrial forms, the usual transition is from an aquatic ancestry to more recent land-living forms. There is no doubt, from a consideration of the facts of structure, that the aquatic water-breathing Arachnids, represented in the past by the Eurypterines and to-day by the sole survivor Limulus, have preceded the terrestrial air-breathing forms of that group. Hence we see at once that the better-known Arachnida form a series leading from Limulus-like aquatic creatures through scorpions, spiders, and harvestmen to the degenerate Acari or mites. The spiders are specialised and reduced in apparent complexity, as compared with the scorpions, but they cannot be regarded as degenerate, since the concentration of structure which occurs in them results in greater efficiency and power than are exhibited by the scorpion. The determination of the relative degree of perfection of organisation attained by two animals compared is difficult when we introduce, as seems inevitable, the question of efficiency and power, and do not confine the question to the perfection of morphological development. We have no measure of the degree of power manifested by various animals, though it would be possible to arrive at some conclusions as to how that "power" should be estimated. It is not possible here to discuss that matter further. We must be content to point out that it seems that

the spiders, the Pedipalps, and other large Arachnids have not been derived from the scorpions directly, but have independently developed from aquatic ancestors, and from one of these independent groups—probably through the harvestmen from the spiders—the Acari have finally resulted.

Leaving that question for consideration in connection with the systematic statement of the characters of the various groups of Arachnida which follows below, it is well now to consider the following question, viz. seeing that Limulus and Scorpio are such highly developed and specialised forms, and that they seem to constitute, as it were, the first and second steps in the series of recognised Arachnida, what do we know, or what are we led to suppose with regard to the more primitive Arachnida from which the Eurypterines and Limulus and Scorpio have sprung? Do we know, in the recent or fossil condition, any such primitive Arachnids? Such a question is not only legitimate, but prompted by the analogy of at least one other great class of Arthropods. The great Arthropod class, the Crustacea, presents to the zoologist at the present day an immense range of forms, comprising the primitive Phyllopods, the minute Copepods, the parasitic Cirrhipedes and the powerful crabs and lobsters, and the highly elaborated sand-hoppers and slaters. It has been insisted, by those who accepted Lankester's original doctrine of the direct or genetic affinity of the Chætopoda and Arthropoda, that Apus and Branchipus really come very near to the ancestral forms which connected those two great branches of Appendiculate (Parapodiate) animals. On the other hand, the land crabs are at an immense distance from these simple forms. The record of the Crustacean family tree is, in fact, a fairly complete one—the lower primitive members of the group are still represented by living forms in great abundance. In the case of the Arachnida, if we have to start their genealogical history with Limulus and Scorpio, we are much in the same position as we should be in dealing with the Crustacea were the whole of the Entomostraca and the whole of the

Arthrostaca wiped out of existence and record. There is no possibility of doubt that the series of forms corresponding in the Arachnidan line of descent to the forms distinguished in the Crustacean line of descent as the lower grade—the Entomostraca—have ceased to exist; and not only so, but have left little evidence in the form of fossils as to their former existence and nature. It must, however, be admitted as probable that we should find some evidence, in ancient rocks or in the deep sea, of the early more primitive Arachnids. And it must be remembered that such forms must be expected to exhibit, when found, differences from Limulus and Scorpio as great as those which separate Apus and Cancer. The existing Arachnida, like the higher Crustacea, are "nomomeristic,"—that is to say, have a fixed typical number of somites to the body. Further, they are like the higher Crustacea, "somatotagmic,"—that is to say, they have this limited set of somites grouped in three (or more) "tagmata," or regions of a fixed number of similarly modified somites—each tagma differing in the modification of its fixed number of somites from that characterising a neighbouring "tagma." The most primitive among the lower Crustacea, on the other hand, for example the Phyllopoda, have not a fixed number of somites; some genera—even allied species—have more, some less, within wide limits; they are "anomomeristic." They also, as is generally the case with anomomeristic animals, do not exhibit any conformity to a fixed plan of "tagmatism," or division of the somites of the body into regions sharply marked off from one another; the head or prosomatic tagma is followed by a trunk consisting of somites which either graduate in character as we pass along the series, or exhibit a large variety in different genera, families, and orders of grouping of the somites. They are anomotagmic as well as anomomeristic.

When it is admitted, as seems to be reasonable, that the primitive Arachnida would, like the primitive Crustacea, be anomomeristic and anomotagmic, we shall not demand of claimants for the rank of primitive Arachnids agreement with

Limulus and Scorpio in respect of the exact number of their somites and the exact grouping of those somites; and when we see how diverse are the modifications of the branches of the appendages, both in Arachnida and in other classes of Arthropoda (*q. v.*), we shall not over-estimate a difference in the form of this or that appendage exhibited by the claimant as compared with the higher Arachnids. With those considerations in mind, the claim of the extinct group of the Trilobites to be considered as representatives of the lower and more primitive steps in the Arachnidan genealogy must, it seems, receive a favourable judgment. They differ from the Crustacea in that they have only a single pair of præoral appendages, the second pair being definitely developed as mandibles. This fact renders their association with the Crustacea impossible, if classification is to be the expression of genetic affinity inferred from structural coincidence. On the contrary, this particular point is one in which they agree with the higher Arachnida. But little is known of the structure of these extinct animals; we are therefore compelled to deal with such special points of resemblance and difference as their remains still exhibit. They had lateral eyes,[1] which resemble no known eyes so closely as the lateral eyes of Limulus. The general form and structure of their prosomatic carapace are in many striking features identical with that of Limulus. The trilobation of the head and body—due to the expansion and flattening of the sides or "pleura" of the tegumentary skeleton—is so closely repeated in the young of Limulus that the latter has been called "the Trilobite stage" of Limulus (Fig. 42 compared with Fig. 41). No Crustacean exhibits this Trilobite form. But most important of the evidences presented by the Trilobites of affinity with Limulus, and therefore with the Arachnida, is the tendency, less marked in some, strongly carried out in others, to form a

[1] A pair of round tubercles on the labrum (camerostome or hypostoma) of several species of Trilobites has been described and held to be a pair of eyes quite recently (**22**). Sense-organs in a similar position were discovered in Limulus by Patten (**42**) in 1894.

pygidial or telsonic shield—a fusion of the posterior somites of the body, which is precisely identical in character with the metasomatic carapace of Limulus. When to this is added the fact that a post-anal spine is developed to a large size in some Trilobites (Fig. 38), like that of Limulus and Scorpio, and that lateral spines on the pleura of the somites are frequent as in Limulus, and that neither metasomatic fusion of somites nor post-anal spine, nor lateral pleural spines are found in any Crustacean, nor all three together in any Arthropod besides the Trilobites and Limulus, the claim of the Trilobites to be considered as representing one order of a lower grade of Arachnida, comparable to the grade Entomostraca of the Crustacea, seems to be established.

The fact that the single pair of præoral appendages of Trilobites, known only as yet in one genus, is in that particular case a pair of uniramose antennæ, does not render the association of Trilobites and Arachnids improbable. Although the præoral pair of appendages in the higher Arachnida is usually chelate, it is not always so; in spiders it is not so; nor in many Acari. The biramose structure of the post-oral limbs, demonstrated by Beecher in the Trilobite Triarthrus, is no more inconsistent with its claim to be a primitive Arachnid than is the foliaceous modification of the limbs in Phyllopods inconsistent with their relationship to the Arthrostracous Crustaceans such as Gammarus and Oniscus.

Thus, then, it seems that we have in the Trilobites the representatives of the lower phases of the Arachnidan pedigree. The simple anomomeristic Trilobite, with its equiformal somites and equiformal appendages, is one term of the series which ends in the even more simple but degenerate Acari. Between the two and at the highest point of the arc, so far as morphological differentiation is concerned, stands the scorpion; near to it in the Trilobite's direction (that is on the ascending side) are Limulus and the Eurypterines—with a long gap, due to obliteration of the record, separating them from the Trilobite. On the other side—tending downwards from the scorpion towards the Acari—are the Pedipalpi, the

spiders, the book-scorpions, the harvestmen, and the water-mites.

The strange Nobody-Crabs or Pycnogonids occupy a place on the ascending half of the arc below the Eurypterines and Limulus. They are strangely modified and degenerate, but seem to be (as explained in the systematic review) the remnant of an Arachnidan group holding the same relation to the scorpions which the Læmodipoda hold to the Podophthalmate Crustacea.

We have now to offer a classification of the Arachnida, and to pass in review the larger groups, with a brief statement of their structural characteristics.

In the bibliography at the close of this article (referred to by leaded Arabic numerals in brackets throughout these pages) the titles of works are given which contain detailed information as to the genera and species of each order or sub-order, their geographical distribution, and their habits and economy so far as they have been ascertained. The limits of space do not permit of a fuller treatment of those matters here.

Tabular Classification[1] of the Arachnida.

Class ARACHNIDA.

Grade A. ANOMOMERISTICA.

Sub-class TRILOBITÆ.

Orders. Not satisfactorily determined.

[1] The writer is indebted to Mr. R. I. Pocock, assistant in the Natural History departments of the British Museum, for valuable assistance in the preparation of this article and for the classification and definition of the groups of Eu-arachnida here given. The general scheme and some of the details have been brought by the writer into agreement with the views maintained in this article. Mr. Pocock accepts those views in all essential points, and has, as a special student of the Arachnida, given to them valuable expansion and confirmation.

Grade B. NOMOMERISTICA.

Sub-class I. **PANTOPODA.**

Order 1. **Nymphonomorpha.**

„ 2. **Ascorhynchomorpha.**

„ 3. **Pycnogonomorpha.**

Sub-class II. **EUARACHNIDA.**

Grade *a*. DELOBRANCHIA, Lankester (vel HYDROPNEUSTEA, Pocock).

Order 1. **Xiphosura.**

„ 2. **Gigantostraca.**

Grade *b*. EMBOLOBRANCHIA, Lankester (vel AËROPNEUSTEA, Pocock).

Section *a*. Pectinifera.

Order 1. **Scorpionidea.**

Sub-order *a*. Apoxypoda.

„ *b*. Dionychopoda.

Section β. Epectinata.

Order 2. **Pedipalpi.**

Sub-order *a*. Uropygi.

Tribe 1. Urotricha.

„ 2. Tartarides.

Sub-order *b*. Amblypygi.

Order 3. **Araneæ.**

Sub-order *a*. Mesothelæ.

„ *b*. Opisthothelæ.

Tribe 1. Mygalomorphæ.

„ 2. Arachnomorphæ.

Order 4. **Palpigradi** (= **Microthelyphonida**).

Order 5. **Solifugæ** (= **Mycetophoræ**).

Order 6. **Pseudoscorpiones** (= **Chelonethi**).

Sub-order *a*. Panctenodactyli.

„ *b*. Hemictenodactyli.

Order 7. **Podogona** (= **Meridogastra**).

Order 8. **Opiliones.**

Sub-order *a*. Cyphophthalmi.

„ *b*. Mecostethi.

„ *c*. Plagiostethi.

Order 9. Rhynchostomi (=Acari).

Sub-order *a*. Notostigmata.
„ *b*. Cryptostigmata.
„ *c*. Metastigmata.
„ *d*. Prostigmata.
„ *e*. Astigmata.
„ *f*. Vermiformia.
„ *g*. Tetrapoda.

Class ARACHNIDA.—Euarthropoda having two prosthomeres (somites which have passed from a post-oral to a præoral position), the appendages of the first represented by eyes, of the second by solitary rami which are rarely antenniform, more usually chelate. A tendency is exhibited to the formation of a metasomatic as well as a prosomatic carapace by fusion of the tergal surfaces of the somites. Intermediate somites forming a mesosoma occur, but tend to fuse superficially with the metasomatic carapace or to become coordinated with the somites of the metasoma, whether fused or distinct to form one region—the opisthosoma (abdomen of authors). In the most highly developed forms the two anterior divisions (tagmata) of the body, prosoma and mesosoma, each exhibit six pairs of limbs, pediform and platelike respectively, whilst the metasoma consists of six limbless somites and a post-anal spine. The genital apertures are placed in the first somite following the prosoma, excepting where a prægenital somite, usually suppressed, is retained. Little is known of the form of the appendages in the lowest archaic Arachnida, but the tendency of those of the prosomatic somites has been (as in the Crustacea) to pass from a generalised biramose or multiramose form to that of uniramose antennæ, chelæ, and walking legs.

The Arachnida are divisible into two grades of structure—according to the fixity or non-fixity of the number of somites building up the body.

Grade A (of the Arachnida). ANOMOMERISTICA.—Extinct archaic Arachnida in which (as in the Entomostracous Crustacea) the number of well-developed

somites may be more or less than eighteen, and may be grouped only as head (prosoma) and trunk, or may be further differentiated. A telsonic tergal shield of greater or less size is always present, which may be imperfectly divided into well-marked but immovable tergites indicating incompletely

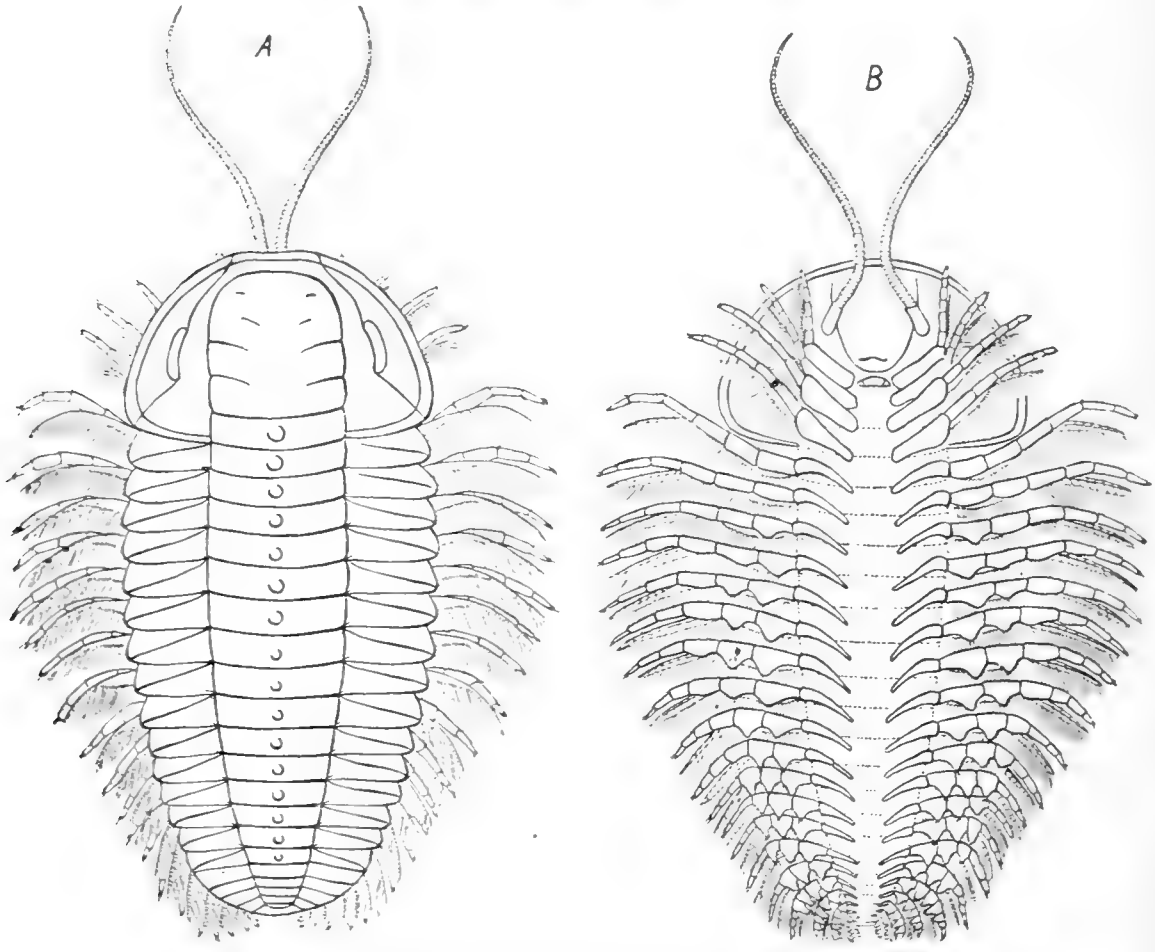


FIG. 34.—Restoration of Triasthrus Becki, Green, as determined by Mr. Beecher from specimens obtained from the Utica Slates (Ordovician), New York. A, dorsal; B, ventral surface. In the latter the single pair of antennæ springing up from each side of the camerostome or hypostome or upper lip-lobe are seen. Four pairs of appendages besides these are seen to belong to the cephalic tergum. All the appendages are pediform and biramose; all have a prominent gnathobase, and in all the exopodite carries a comb-like series of secondary processes. (After Beecher, from Zittel.)

differentiated somites. The single pair of palpiform appendages in front of the mouth has been found in one instance to be antenniform, whilst the numerous post-oral appendages in

the same genus were biramose. The position of the genital apertures is not known. Compound lateral eyes present; median eyes wanting. The body and head have the two pleural regions of each somite flattened and expanded on either side of the true gut-holding body-axis. Hence the name of the sub-class signifying trilobed, a condition realised also in the Xiphosurous Arachnids. The members of this group, whilst resembling the lower Crustacea (since all lower groups of a phylum tend to resemble one another), differ from them essentially in that the head exhibits only one prosthomere (in addition to the eye-bearing prosthomere) with palpi-

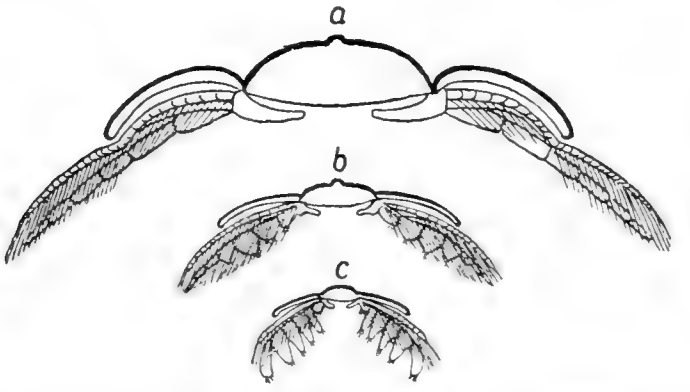


FIG. 35.—Triarthrus Becki, Green. *a*, Restored thoracic limbs in transverse section of the animal: *b*, section across a posterior somite; *c*, section across one of the sub-terminal somites. (After Beecher.)

form appendages (as in all Arachnida) instead of two. The Anomomeristic Arachnida form a single sub-class, of which only imperfect fossil remains are known.

Sub-class (of the Anomomeristica) TRILOBITÆ.—The single sub-class Trilobitæ constitutes the grade Anomomeristica. It has been variously divided into orders by a number of writers. The greater or less evolution and specialisation of the metasomatic carapace appears to be the most important basis for classification—but this has not been made use of in the latest attempts at drawing up a system of the Trilobites. The form of the middle and lateral regions of the prosomatic shield has been used, and an excessive importance attached to the

demarcation of certain areas in that structure. Sutures are stated to mark off some of these pieces, but in the proper sense of that term, as applied to the skeletal structures of the Vertebrata, no sutures exist in the chitinous cuticle of Arthropoda. That any partial fusion of originally distinct chitinous plates takes place in the cephalic shield of Trilobites, comparable to the partial fusion of bony pieces by suture in Vertebrata, is a suggestion contrary to fact.

The Trilobites are known only as fossils, mostly Silurian and pre-Silurian; a few are found in Carboniferous and Permian strata. As many as two thousand species are known. Genera

Fig. 36.

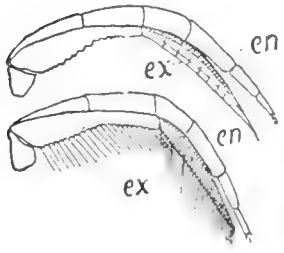


Fig. 37.

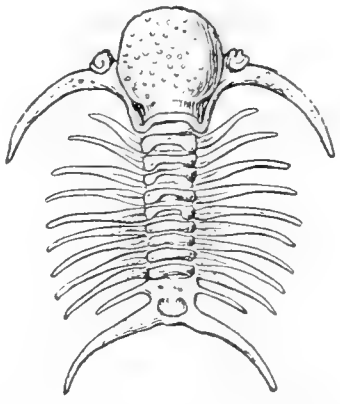


Fig. 36.—Triarthrus Becki, Green. Dorsal view of second thoracic leg with and without setæ. *en*, inner ramus; *ex*, outer ramus. (After Beecher.)

Fig. 37.—Deiphon Forbesii, Barr. One of the Cheiruridæ. Silurian, Bohemia. (From Zittel's 'Palæontology.)

with small metasomatic carapace, consisting of three to six fused segments distinctly marked though not separated by soft membrane, are Harpes, Paradoxides, and Triarthrus (Fig. 34). In Calymene, Homalonotus, and Phacops (Fig. 38) from six to sixteen segments are clearly marked by ridges and grooves in the metasomatic tagma, whilst in Ilænus (Fig. 39) the shield so formed is large, but no somites are marked out on its surface. In this genus ten free somites (mesosoma) occur between the prosomatic and metasomatic carapaces. Asaphus and Megalaspis (Fig. 39) are similarly constituted. In Agnos-

tus (Fig. 40) the anterior and posterior carapaces constitute almost the entire body, the two carapaces being connected by a mid-region of only two free somites. It has been held that the forms with a small number of somites marked in the posterior carapace, and numerous free somites between the anterior and posterior carapace, must be considered as anterior to those in which a great number of posterior somites are

FIG. 38.

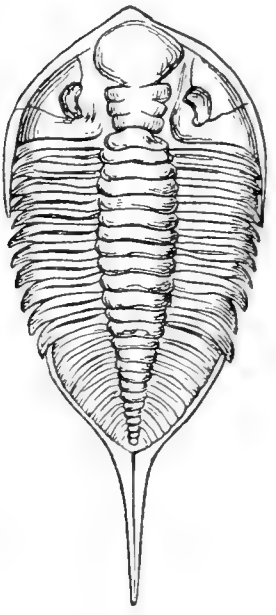


FIG. 39.

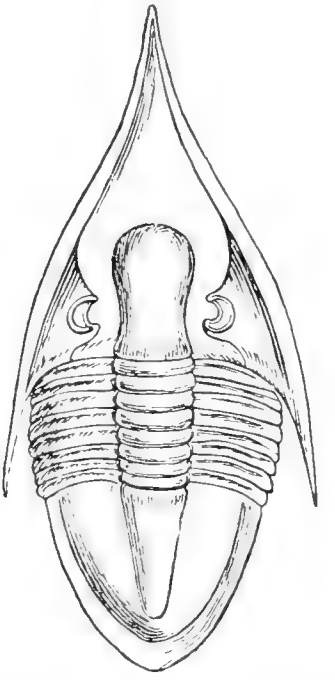


FIG. 38.—Dalmanites (Phacops) limulurus, Green. One of the Phacopidæ, from the Silurian, New York. (From Zittel.)

FIG. 39.—Megalaspis extenuatus. One of the Asaphidæ allied to Ilænus, from the Ordovician of East Gothland, Sweden. (From Zittel.)

traceable in the metasomatic carapace, and that those in which the traces of distinct somites in the posterior or metasomatic carapace are most completely absent must be regarded as derived from those in which somites are well marked in the posterior carapace and similar in appearance to the free somites. The genus Agnostus, which belongs to the last category, occurs abundantly in Cambrian strata, and is one of the earliest forms known. This would lead to the supposi-

tion that the great development of metasomatic carapace is a primitive and not a late character, were it not for the fact that Paradoxides and Atops, with an inconspicuous telsonic carapace and numerous free somites, are also Cambrian in age, the latter, indeed, anterior in horizon to Agnostus.

On the other hand, it may well be doubted whether the pygidial or posterior carapace is primarily due to a fusion of the tergites of somites which were previously movable and well developed. The posterior carapace of the Trilobites and of Limulus is probably enough in origin a telsonic carapace—that is to say, is the tergum of the last segment of the body

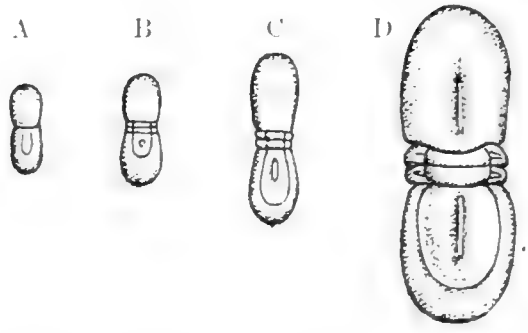


FIG. 40.—Four stages in the development of the trilobite Agnostus nudus. A, youngest stage with no mesosomatic somites. B and C, stages with two mesosomatic somites between the prosomatic and telsonic carapaces; D, adult condition, still with only two free mesosomatic somites. (From Korschelt and Heider.)

which carries the anus. From the front of this region new segments are produced in the first instance, and are added during growth to the existing series. This telson may enlarge, it may possibly even become internally and sternally developed as partially separate somites, and the tergum may remain without trace of somite formation, or, as appears to be the case in Limulus, the telson gives rise to a few well-marked somites (mesosoma and two others), and then enlarges without further trace of segmentation, whilst the chitinous integument which develops in increasing thickness on the terga as growth advances welds together the unsegmented telson and the somites in front of it, which were

previously marked by separate tergal thickenings. It must always be remembered that we are liable (especially in the case of fossilised integuments) to attach an unwarranted interpretation to the mere discontinuity or continuity of the thickened plates of chitinous cuticle on the back of an Arthropod. These plates may fuse, and yet the somites to which they belong may remain distinct, and each have its pair of

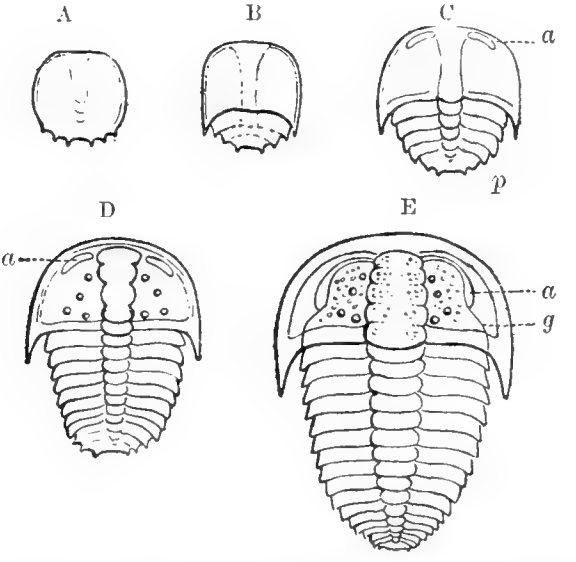


FIG. 41.—Five stages in the development of the trilobite Sao hirsuta. A, youngest stage; B, older stage with distinct pygidial carapace; C, stage with two free mesosomatic somites between the prosomatic and telsonic carapaces; D, stage with seven free intermediate somites; E, stage with twelve free somites; the telsonic carapace has not increased in size; *a*, lateral eye; *g*, so-called facial "suture" (not really a suture); *p*, telsonic carapace. (From Korschelt and Heider, after Barrande.)

appendages well developed. On the other hand, an unusually large tergal plate, whether terminal or in the series, is not always due to fusion of the dorsal plates of once-separated somites, but is often a case of growth and enlargement of a single somite without formation of any trace of a new somite. For the literature of Trilobites see 22*.

Grade B (of the Arachnida). NOMOMERISTICA.—Arachnida in which, excluding from consideration the eye-bearing prosthomere, the somites are primarily (that is to say,

in the common ancestor of the grade) grouped in three regions of six—(*a*) the "prosoma" with palpiform appendages, (*b*) the "mesosoma" with plate-like appendages, and (*c*) the "metasoma" with suppressed appendages. A somite placed between the prosoma and mesosoma—the pre-genital somite—appears to have belonged originally to the prosomatic series (which with its ocular prosthomere and palpiform limbs [Pantopoda] would thus consist of eight somites), but to have been gradually reduced. In living Arachnids, excepting the Pantopoda, it is either fused (with loss of its appendages) with the prosoma (Limulus,[1] Scorpio), after embryonic appearance, or is retained as a rudimentary,

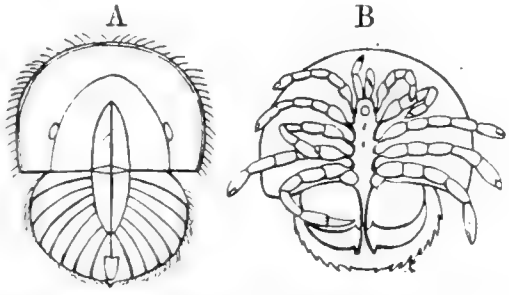


FIG. 42.—So-called "trilobite stage" of Limulus polyphemus. A, dorsal, B, ventral view. (From Korschelt and Heider, after Leuckart.)

separate, detached somite in front of the mesosoma, or disappears altogether (excalation). The atrophy and total disappearance of ancestrally well-marked somites frequently take place (as in all Arthropoda) at the posterior extremity of the body, whilst excalation of somites may occur at the constricted areas which often separate adjacent "regions," though there are very few instances in which it has been recognised. Concentration of the organ-systems by fusion of neighbouring regions (prosoma, mesosoma, metasoma), pre-

[1] Mr. Pocock suggests that the area marked vii in the outline figure of the dorsal view of Limulus (Fig. 7) may belong to the tergum of the suppressed pregenital somite. A small area on the prosomatic carapace (marked * in fig. 7) is also considered by Mr. Pocock as possibly belonging to the pre-genital somite, and this latter suggestion is what commends itself to the present writer. Embryological evidence must settle exactly what has become of the pre-genital somite.—E. R. L.

viously distinct, has frequently occurred, together with obliteration of the muscular and chitinous structures indicative of distinct somites. This concentration and obliteration of somites, often occompanied by dislocation of important segmental structures (such as appendages and nerve-ganglia), may lead to highly-developed specialisation (individuation, H. Spencer), as in the Araneæ and Opiliones; and, on the other hand, may terminate in simplification and degeneration, as in the Acari.

The most important general change which has affected the structure of the nomomeristic Arachnida in the course of their historic development is the transition from an aquatic to a terrestrial life. This has been accompanied by the conversion of the lamelliform gill-plates into lamelliform lung-plates, and later the development from the lung-chambers, and at independent sites, of tracheæ or air-tubes (by adaptation of the vasifactive tissue of the blood-vessels) similar to those independently developed in Peripatus, Diplopoda, Hexapoda, and Chilopoda. Probably tracheæ have developed independently by the same process in several groups of tracheate Arachnids. The nomomeristic Arachnids comprise two sub-classes—one a very small degenerate offshoot from early ancestors, the other the great bulk of the class.

Sub-class I (of the Nomomeristica). PANTOPODA.—Nomomeristic Arachnids in which the somites corresponding to mesosoma and metasoma have entirely aborted. The seventh leg-bearing somite (the pre-genital rudimentary somite of Euarachnida) is present, and has its leg-like appendages fully developed. Monomeniscous eyes with a double (really triple) cell layer formed by invagination, as in the Euarachnida, are present. The Pantopoda stand in the same relation to Limulus and Scorpio that Cyamus holds to the thoracostracous Crustacea. The reduction of the organism to seven leg-bearing somites, of which the first pair, as in so many Euarachnida, are chelate, is a form of degeneration connected with a peculiar quasi-parasitic habit resembling that of the Crustacean Læmodipoda. The genital pores are situate at

the base of the seventh pair of limbs, and may be repeated on the fourth, fifth, and sixth. In all known Pantopoda the size of the body is quite minute as compared with that of the limbs: the alimentary canal sends a long cæcum into each leg (cf. the Araneæ), and the genital products are developed in gonocœls also placed in the legs.

The Pantopoda are divided into three orders, the characters of which are dependent on variation in the presence of the full number of legs.

Order 1 (of the Pantopoda). Nymphonomorpha, Pocock (nov.) (Fig. 43).—In primitive forms belonging to the family Nym-

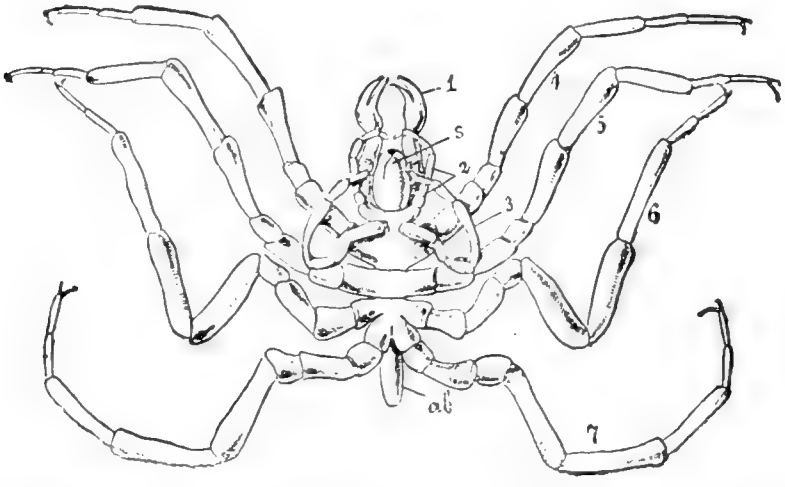


Fig. 43.—One of the Nymphonomorphous Pantopoda, Nymphon hispidum, showing the seven pairs of appendages 1 to 7; *ab*, the rudimentary opisthosoma; *s*, the mouth-bearing proboscis. (From Parker and Haswell's 'Text-book of Zoology, after Hoek.)

phonidæ the full complement of appendages is retained—the first (mandibular), the second (palpiform), and the third (ovigerous) pairs being well developed in both sexes. In certain derivative forms constituting the family Pallenidæ, however, the appendages of the second pair are either rudimentary or atrophied altogether.

Two families: 1. Nymphonidæ (genus Nymphon), and 2. Pallenidæ (genus Pallene).

Order 2. Ascorhynchomorpha, Pocock (nov.).—Appendages of the second and third pairs retained and developed, as in

the more primitive types of Nymphonomorpha; but those of the first pair are either rudimentary, as in the Ascorhynchidæ, or atrophied, as in the Colossendeidæ. In the latter a further specialisation is shown in the fusion of the body segments.

Two families: 1. Ascorhynchidæ (genera Ascorhynchus and Ammothea); 2. Colossendeidæ (genera Colossendeis and Discoarachne).

Order 3. Pycnogonomorpha, Pocock (nov.).—Derivative forms in which the reduction in number of the anterior appendages is carried farther than in the other orders, reaching its extreme in the Pycnogonidæ, where the first and second pairs are absent in both sexes, and the third pair also are absent in the female. In the Hannoniidæ, however, which resemble the Pycnogonidæ in the absence of the third pair in the female, and of the second pair in both sexes, the first pair are retained in both sexes.

Two families: 1. Hannoniidæ (genus Hannonia); 2. Pycnogonidæ (genera Pycnogonum and Phoxichilus).

Remarks.—The Pantopoda are not known in the fossil condition. They are entirely marine, and are not uncommon in the coralline zone of the sea-coast. The species are few, not more than fifty (**23**). Some large species of peculiar genera are taken at great depths. Their movements are extremely sluggish. They are especially remarkable for the small size of the body and the extension of viscera into the legs. Their structure is eminently that of degenerate forms. Many frequent growths of coralline Algæ and Hydroid polyps, upon the juices of which they feed, and in some cases a species of gall is produced in Hydroids by the penetration of the larval Pantopoda into the tissues of the polyp.

Sub-class II (of the Nomomeristic Arachnida). EUARACHNIDA.—These start from highly developed and specialised aquatic branchiferous forms, exhibiting prosoma with six pediform pairs of appendages, an intermediate prægenital somite, a mesosoma of six somites bearing lamelliform pairs of appendages, and a metasoma of six somites devoid of appendages, and the last provided with a post-anal spine.

Median eyes are present, which are monomeniscous, with distinct retinal and corneagenous cell layers, and placed centrally on the prosoma. Lateral eyes also may be present, arranged in lateral groups, and having a single or double cell layer beneath the lens. The first pair of limbs is often chelate or prehensile, rarely antenniform; whilst the second, third, and fourth may also be chelate, or may be simple palps or walking legs.

An internal skeletal plate, the so-called "entosternite" of fibro-cartilaginous tissue, to which many muscles are attached, is placed between the nerve-cords and the alimentary tract in the prosoma of the larger forms (Limulus, Scorpio, Mygale). In the same and other leading forms a pair of much-coiled glandular tubes, the coxal glands (cœlomocœls in origin), is found with a duct opening on the coxa of the fifth pair of appendages of the prosoma. The vascular system is highly developed (in the non-degenerate forms); large arterial branches closely accompany or envelop the chief nerves; capillaries are well developed. The blood-corpuscles are large amœbiform cells, and the blood-plasma is coloured blue by hæmocyanin.

The alimentary canal is uncoiled and cylindrical, and gives rise laterally to large gastric glands, which are more than a single pair in number (two to six pairs), and may assume the form of simple cæca. The mouth is minute, and the pharynx is always suctorial, never gizzard-like. The gonadial tubes (gonocœls or gonadial cœlom) are originally reticular and paired, though they may be reduced to a simpler condition. They open on the first somite of the mesosoma. In the numerous degenerate forms simplification occurs by obliteration of the demarcations of somites and the fusion of body-regions, together with a gradual suppression of the lamelliferous respiratory organs and the substitution for them of tracheæ, which, in their turn, in the smaller and most reduced members of the group, may also disappear.

The Euarachnida are divided into two grades with refer-

ence to the condition of the respiratory organs as adapted to aquatic or terrestrial life.

Grade *a* (of the Euarachnida). DELOBRANCHIA (Hydropneustea).

Mesosomatic segments furnished with large plate-like appendages, the first pair acting as the genital operculum,

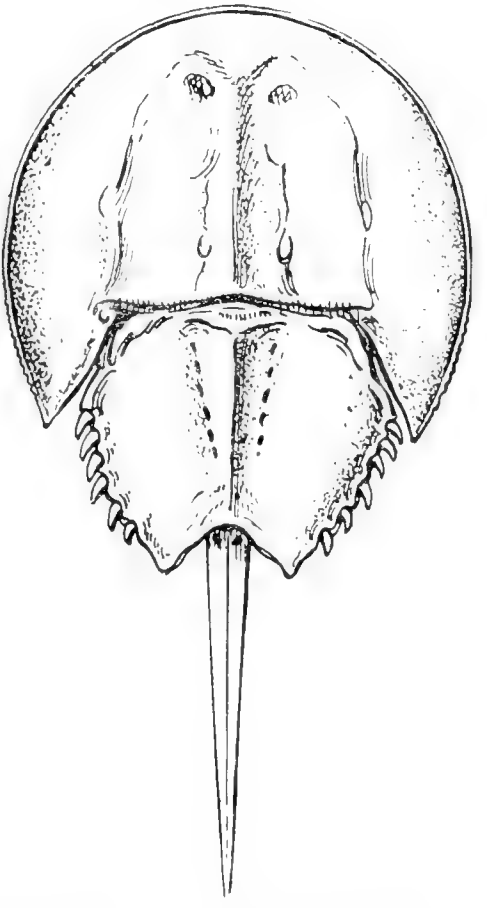


FIG. 44.—Dorsal view of Limulus polyphemus, Lim. One fourth the Natural size, linear. (From Parker and Haswell, 'Text-book of Zoology,' after Leuckart.)

the remaining pairs being provided with branchial lamellæ fitted for breathing oxygen dissolved in water. The præ-genital somite partially or wholly obliterated in the adult. The mouth lying far back, so that the basal segments of all the prosomatic appendages, excepting those of the first pair,

are capable of acting as masticatory organs. Lateral eyes consisting of a densely packed group of eye-units ("compound" eyes).

Order 1. Xiphosura.—The prægenital somite fuses in the

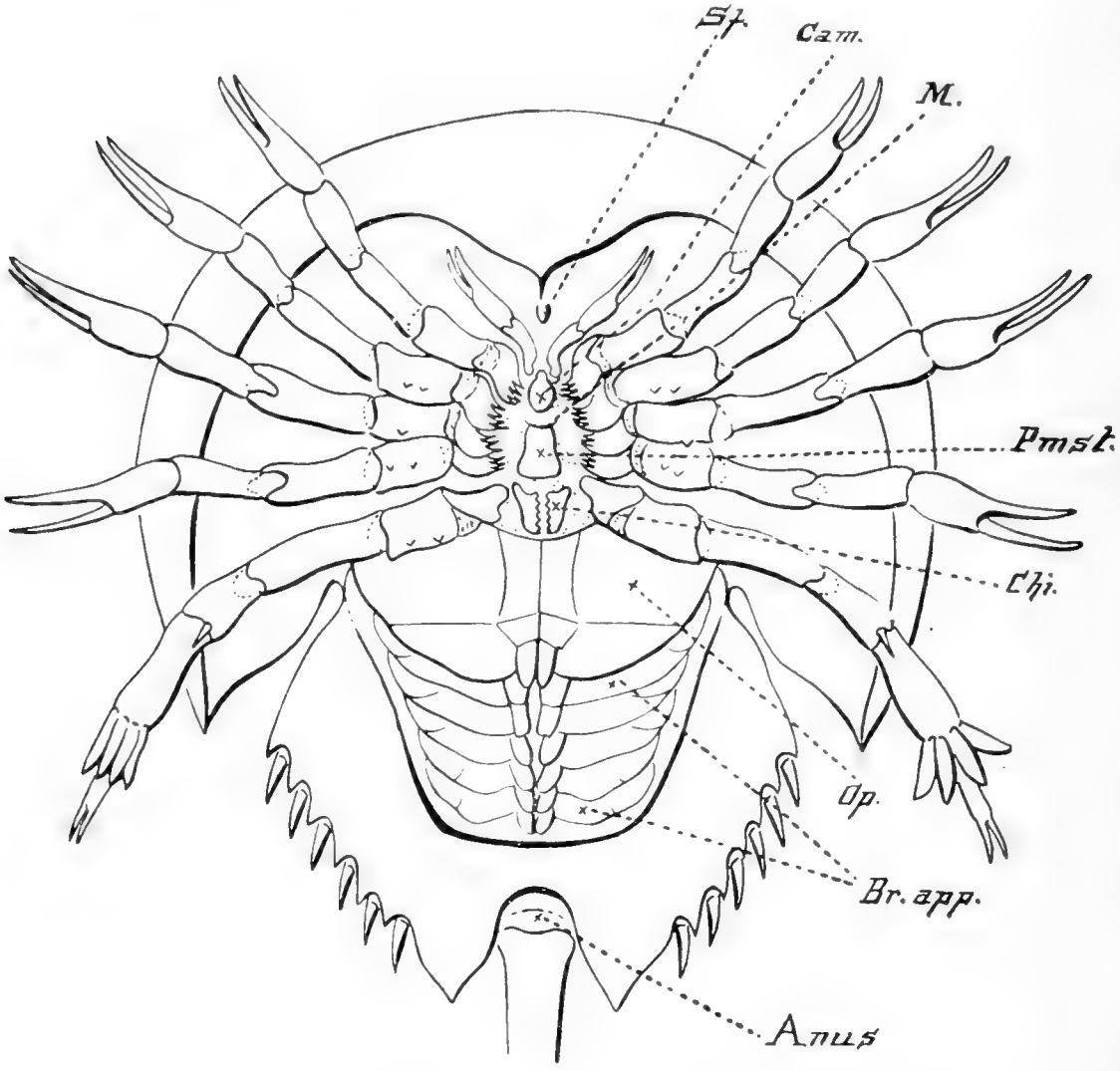


FIG. 45.—Ventral view of Limulus polyphemus, Lim. *Sf*, Subfrontal sclerite; *Cam*, camarostome; *M*, mouth; *Pmst*, promesosternum; *chi*, chilaria; *op*, genital operculum or first pair of appendages of the mesosoma; *Br.app*, second to the sixth pair of appendages of the mesosoma bearing the branchial laminæ.

embryo with the prosoma and disappears (see Fig. 19). Not free-swimming, none of the prosomatic appendages modified to act as paddles; segments of the mesosoma and metasoma

(=opisthosoma) not more than ten in number, distinct or coalesced.

Family—Limulidæ (Limulus).

,, Belinuridæ (Belinurus, Aglaspis, Prestwichia).

,, Hemiaspidæ (Hemiaspis, Bunodes).

Remarks.—The Xiphosura are marine in habit, frequenting the shore. They are represented at the present day by the single genus Limulus (Figs. 44 and 45; also Figs. 7, 9, 11, to 15 and 20), which occurs on the America coast of the Atlantic Ocean, but not on its eastern coasts, and on the Asiatic coast of the Pacific. The Atlantic species (L. polyphemus) is common on the coasts of the United States, and is known as the king-crab or horseshoe crab. A single specimen was found in the harbour of Copenhagen in the eighteenth century, having presumably been carried over by a ship to which it clung.

A species of Limulus is found in the Buntersandstein of the Vosges; L. Walchi is abundant in the Oolitic lithographic slates of Bavaria.

The genera Belinurus, Aglaspis, Prestwichia, Hemiaspis, and Bunodes consist of small forms which occur in Palæozoic rocks. In none of them are the appendages known, but in the form of the two carapaces and the presence of free somites they are distinctly intermediate between Limulus and the Trilobitæ. The young form of Limulus itself (Fig. 40) is also similar to a Trilobite so far as its segmentation and trilobation are concerned. The lateral eyes of Limulus appear to be identical in structure and position with those of certain Trilobitæ.

Order 2. Gigantostraca (Figs. 46, 47).—Free-swimming forms, with the appendages of the sixth or fifth and sixth pairs flattened or lengthened to act as oars; segments of mesosoma and metasoma (= opisthosoma) twelve in number.

Appendages of anterior pair very large and chelate.

Sub-order Pterygotomorpha, Pterygotidæ (Pterygotus).

Appendages of anterior pair minute and chelate.

Sub-order Eurypteromorpha { Stylonuridæ (Stylonurus). Eurypteridæ (Eurypterus, Slimonia).

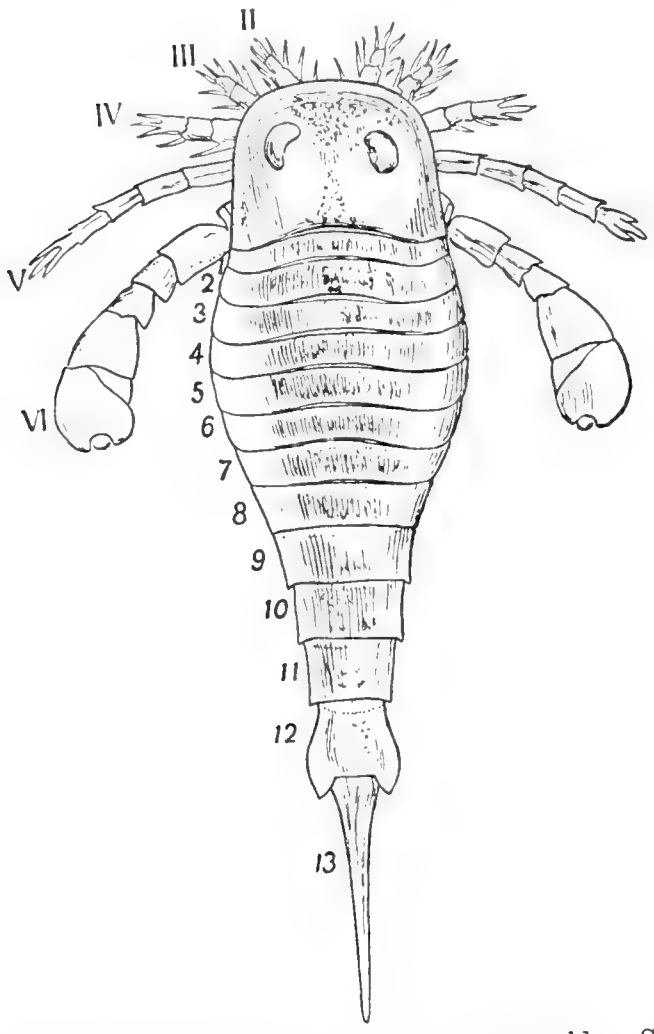


FIG. 46.—Eurypterus Fischeri, Eichwald. Silurian of Rootzikil. Restoration after Schmidt half the size of nature. The dorsal aspect is presented, showing the prosomatic shield, with paired compound eyes, and the prosomatic appendages II to VI. The small first pair of appendages is concealed from view by the carapace. 1 to 12 are the somites of the opisthosoma; 13, the post-anal spine. (From Zittel's 'Text-book of Palæontology.' Macmillans, New York, 1896.)

Remarks.—The Gigantostraca are frequently spoken of as "the Eurypterines." Not more than thirty species are

known. They became extinct in Palæozoic times, and are chiefly found in the Upper Silurian, though extending upwards as far as the Carboniferous. They may be regarded as "macrourous" Xiphosura; that is to say, Xiphosura in which the nomomeristic number of eighteen well-developed somites is present, and the posterior ones form a long tail-like region of the body. There still appears to be some doubt

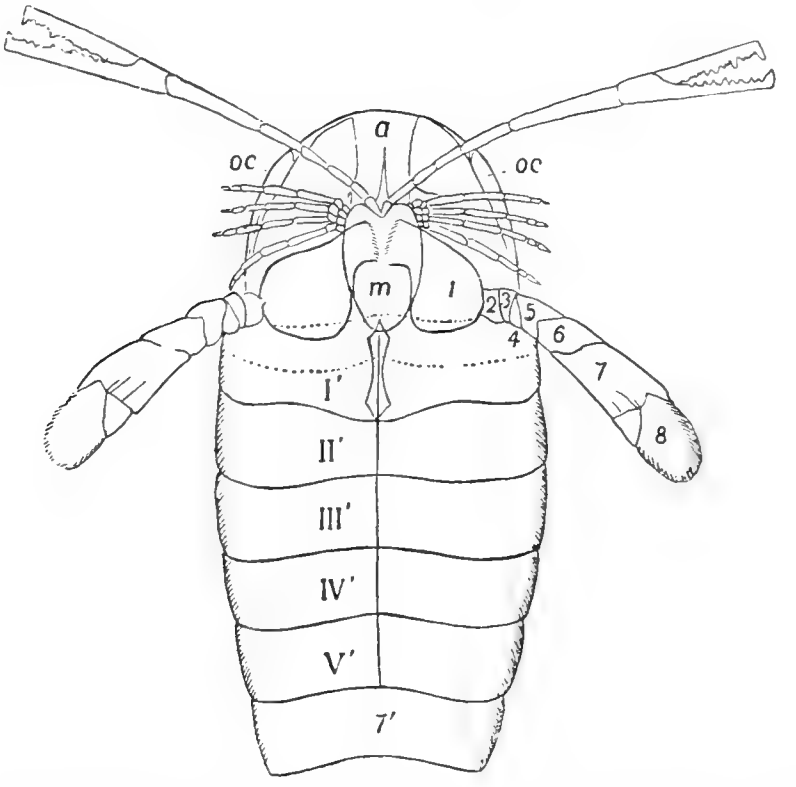


FIG. 47.—Pterygotus osiliensis, Schmidt. Silurian of Rootzikil. Restoration of the ventral surface, one third the natural size, after Schmidt. *a*, camerostome or epistoma; *m*, chilariam or metasternite of the prosoma (so-called metastoma); *oc*, the compound eyes; 1 to 8, segments of the sixth prosomatic appendage; I′ to V′, first five opisthosomatic somites; 7′, sixth opisthosomatic somite. Observe the powerful gnathobases of the sixth pair of prosomatic limbs and the median plates behind *m*. The dotted line on somite I indicates the position of the genital operculum, which was probably provided with branchial lamellæ. (From Zittel's 'Palæontology.')

whether in the sub-order Eurypteromorpha the first pair of prosomatic appendages (Fig. 46) is atrophied, or whether, if present, it has the form of a pair of tactile palps or of minute

chelæ. Though there are indications of lamelliform respiratory appendages on mesomatic somites following that bearing the genital operculum, we cannot be said to have any proper knowledge as to such appendages, and further evidence with regard to them is much to be desired. (For literature see Zittel, 22*.)

Grade *b* (of the Euarachnida). Embolobranchia (Aëropneustea).

In primitive forms the respiratory lamellæ of the appendages of the third, fourth, fifth, and sixth, or of the first and second mesosomatic somites are sunk beneath the surface of the body, and become adapted to breathe atmospheric oxygen, forming the leaves of the so-called lung-books. In specialised forms these pulmonary sacs are wholly or partly replaced by tracheal tubes. The appendages of the mesosoma generally suppressed; in the more primitive forms one or two pairs may be retained as organs subservient to reproduction or silk-spinning. Mouth situated more forwards than in Delobranchia, no share in mastication being taken by the basal segments of the fifth and sixth pairs of prosomatic appendages. Lateral eyes, when present, represented by separate ocelli.

The prægenital somite, after appearing in the embryo, either is obliterated (Scorpio, Galeodes, Opileo, and others) or is retained as a reduced narrow region of the body, the "waist," between prosoma and mesosoma. It is represented by a full-sized tergal plate in the pseudo-Scorpiones.

Section *a*. Pectinifera.—The primitive distinction between the mesosoma and the metasoma retained, the latter consisting of six somites and the former of six somites in the adult, each of which is furnished during growth with a pair of appendages. Including the prægenital somite (fig. 16), which is suppressed in the adult, there are thirteen somites behind the prosoma. The appendages of the first and second mesosomatic somites persisting as the genital operculum and

pectones respectively, those of the third, fourth, fifth, and sixth somites (? in Palæophonus) sinking below the surface during growth in connection with the formation of the four pairs of pulmonary sacs (see Fig. 17). Lateral eyes monostichous.

Fig. 48.

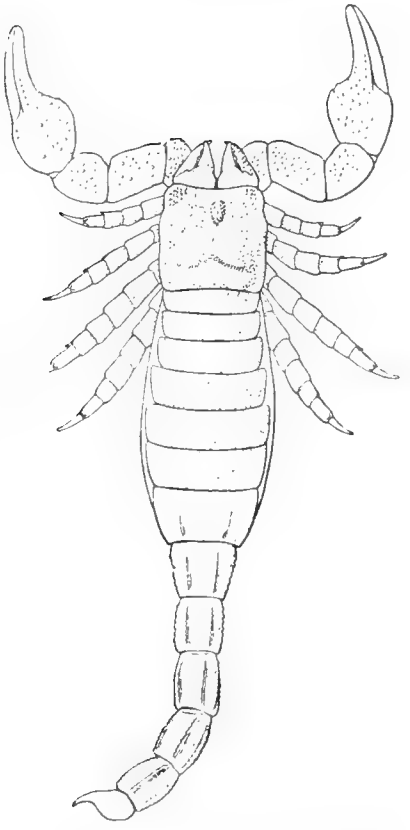


Fig. 49.

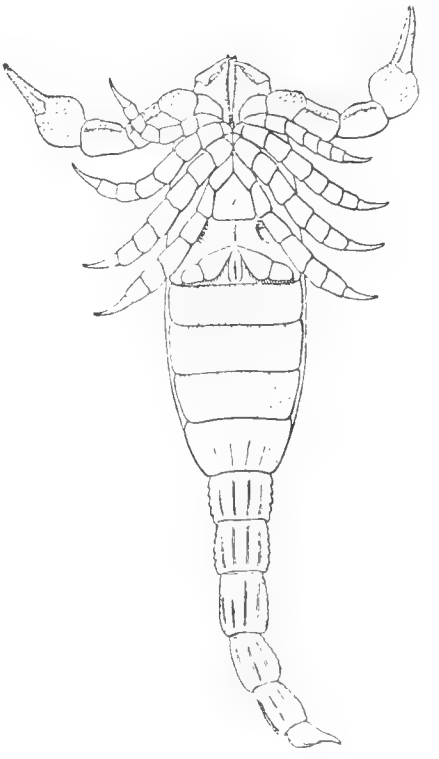


Fig. 48.—Dorsal view of a restoration of Palæophonus nuncius, Thorell, the Silurian Scorpion from Gothland. (Restored after Thorell's indications by Mr. R. I. Pocock.)

Fig. 49.—Ventral view of a restoration of Palæophonus Hunteri, Pocock, the Silurian Scorpion from Lesmahago, Scotland. Restored by Mr. R. I. Pocock. The meeting of the coxæ of all the prosomatic limbs in front of the pentagonal sternum; the space for a genital operculum; the pair of pectens, and the absence of any evidence of pulmonary stigmata are noticeable in this specimen. (See Pocock,' Quart. Journ. Micr. Sci.,' 1901.)

Order 1. Scorpionidea.—Prosoma covered by a single dorsal shield, bearing typically median and lateral eyes; its sternal elements reduced to a single plate lodged between or behind

the basal segments of the fifth and sixth pairs of appendages. Appendages of first pair tri-segmented, chelate; of second pair chelate, with their basal segments subserving mastication; of third, fourth, fifth, and sixth pairs similar in form and function, except that in recent and Carboniferous forms the basal segments of the third and fourth are provided with sterno-coxal (maxillary) lobes, those of the fourth pair meeting in the middle line and underlying the mouth. The five posterior somites of the metasoma constricted to form a "tail," the post-anal sclerite persisting as a weapon of offence, and provided with a pair of poison glands (see Figs. 8, 10, 12, 13, 14, 15, 21, 22).

Sub-order Apoxypoda.—The third, fourth, fifth, and sixth pairs of appendages short, stout, tapering, the segments about as wide as long, except the apical, which is distally slender, pointed, slightly curved, and without distinct movable claws.

Family Palæophonidæ, Palæophonus (Figs. 48 and 49).

Sub-order Dionychopoda.—The third, fourth, fifth, and sixth pairs of appendages slender, not evenly tapering, the segments longer than wide; the apical segment short, distally truncate, and provided with a pair of movable claws. Basal segments of the fifth and sixth pairs of appendages abutting against the sternum of the prosoma (see Fig. 10 and Figs. 51, 52, and 53).

Family—Pandinidæ (Pandinus, Opisthophthalmus, Urodacus).

,, Væjovidæ (Væjovis, Iurus, Euscorpius, Broteas).

,, Bothriuridæ (Bothriurus, Cercophonius).

,, Buthidæ (Buthus, Centrurus).

,, *Cyclophthalmidæ (Cyclophthalmus)

,, *Eoscorpiidæ (Eoscorpius, Centromachus)

} Carboniferous.

Remarks on the Order Scorpionidea.—The scorpion is one of the great animals of ancient lore and tradition. It

and the crab are the only two invertebrates which had impressed the minds of early men sufficiently to be raised to the dignity of astronomical representation. It is all the more remarkable that the scorpion proves to be the oldest animal form of high elaboration which has persisted to the present day. In the Upper Silurian two specimens of a scorpion have been found (Figs. 48, 49), one in Gothland and one in Scotland, which would be recognised at once as true scorpions

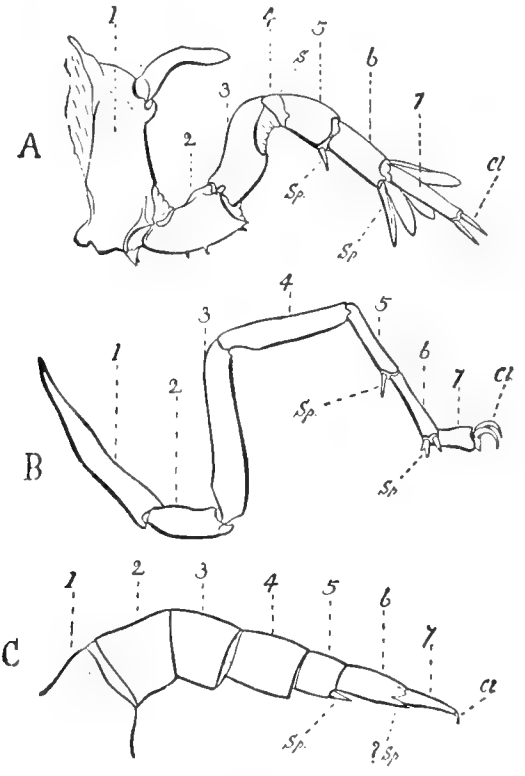


FIG. 50.—Comparison of the sixth prososomatic limb of a recent Scorpion (B), of Palæophonus (C), and of Limulus (A), showing their agreement in the number of segments; in the existence of a movable spine, *Sp*, at the distal border of fifth segment; in the correspondence of the two claws at the free end of the limb of Scorpio with two spines similarly placed in Limulus; and, lastly, in the correspondence of the three talon-like spines carried on the distal margin of segment six of recent Scorpions with the four larger but similarly situated spines on the leg of Limulus; *s*, groove dividing the ankylosed segments 4 and 5 of the Limulus leg into two. (After Pocock, 'Quart. Journ. Micr. Sci.,' 1901.)

by a child or a savage. The Silurian scorpion, Palæophonus, differs, so far as obvious points are concerned, from

a modern scorpion only in the thickness of its legs, and in their terminating in strong spike-like joints, instead of being slight, and provided with a pair of terminal claws. The legs of the modern scorpion (Fig. 10 : Fig. 51) are those of a terrestrial Arthropod, such as a beetle; whilst those of the Silurian scorpion are the legs of an aquatic Arthropod, such as a crab or lobster. It is probable that the Silurian scorpion was an aquatic animal, and that its respiratory lamellæ were still projecting from the surface of the body to serve as branchiæ. No trace of "stigmata," the orifices of the lung-chambers of modern scorpions, can be found in the Scottish

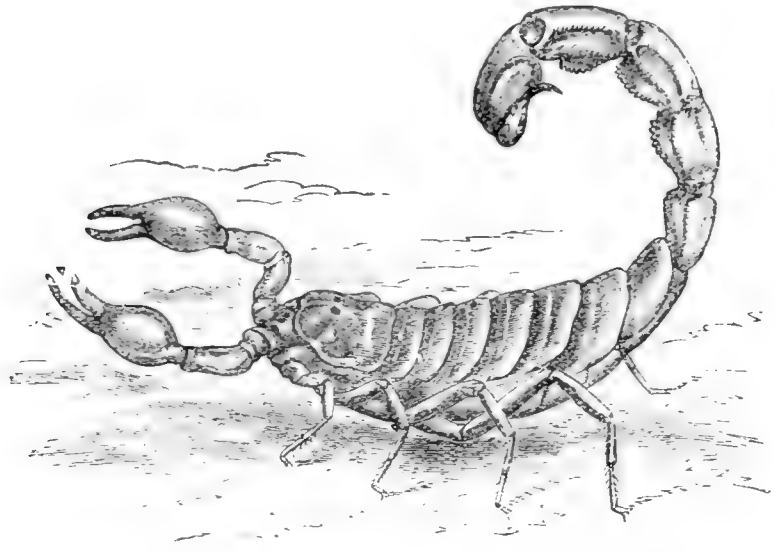


Fig. 51.—Drawing from life of the desert Scorpion, Buthus australis, Lin., from Biskra, N. Africa. (From Lankester, 'Journ. Linn. Soc. Zool.,' vol. xvi, 1881.)

specimen of Palæophonus, which presents the ventral surface of the animal to view. On the other hand, no trace of respiratory appendages, excepting the pectens, can be detected in the specimen (see Fig. 49).

Fossil scorpions of the modern type are found in the Coal Measures. At the present day scorpions of various genera are found in all the warm regions of the world. In Europe they occur as far north as Bavaria and the south of France The largest species measure nine inches from the front of the

head to the end of the sting, and occur in tropical India and Africa. Between 200 and 300 species are known. The scorpions use their large chelæ for seizing prey and for fighting with one another. They never use the sting when (as frequently happens) they attack another scorpion, because, as was ascertained by A. G. Bourne (**24**), the poison exuded by the sting has no injurious effect on another scorpion nor on the scorpion itself. The stories of a scorpion stinging itself to death when placed in a circle of burning coals are due to erroneous observation. When placed in such a position the scorpion faints and becomes inert. It is found (Bourne, **24**) that some species of scorpion faint at a temperature of 40° Cent. They recover on being removed to cooler conditions. A scorpion, having seized its prey (usually a large insect, or small reptile or mammal) with the large chelæ, brings its tail over its head, and deliberately punctures the struggling victim twice with its sting (Fig. 52). The poison of the sting is similar to snake poison (Calmette), and rapidly paralyses animals which are not immune to it. It is probably only sickly adults or young children of the human race who can be actually killed by a scorpion's sting. When the scorpion has paralysed its prey in this way the two short cheliceræ are brought into play (Fig. 53). By the crushing action of their pincers, and an alternate backward and forward movement, they bring the soft blood-holding tissues of the victim close to the minute pin-hole aperture which is the scorpion's mouth. The muscles acting on the bulb-like pharynx now set up a pumping action (see Huxley [**26**]); and the juices—but no solid matter, excepting such as is reduced to powder—are sucked into the scorpion's alimentary canal. A scorpion appears to prefer for its food another scorpion, and will suck out the juices of an individual as large as itself. When this has taken place the gorged scorpion becomes distended and tense in the mesosomatic region. It is certain that the absorbed juices do not occupy the alimentary canal alone, but pass also into its cæcal off-sets, which are the ducts of the gastric glands (see Fig. 33).

All Arachnida, including Limulus, feed by suctorial action in essentially the same way as Scorpio.

Scorpions of various species have been observed to make a hissing noise when disturbed, or even when not disturbed. The sound is produced by stridulating organs developed on the basal joints of the limbs, which differ in position and character in different genera (see Pocock [**27**]). Scorpions copulate with the ventral surfaces in contact. The eggs are fertilised, practically in the ovary, and develop in situ. The

Fig. 53.

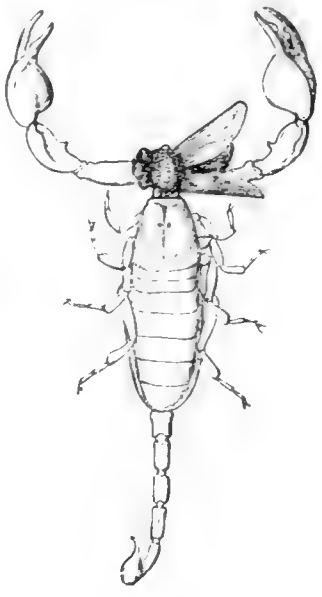


Fig. 52.

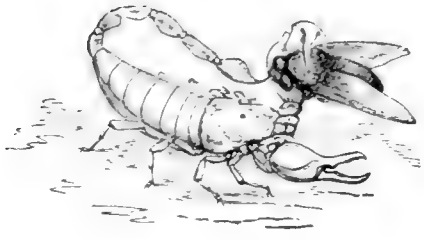


Fig. 52.—Drawing from life of the Italian Scorpion Euscorpius italicus, Herbst, holding a blue-bottle fly with its left chela and carefully piercing it between head and thorax with its sting. Two insertions of the sting are effected, and the fly is instantly paralysed by the poison so introduced into its body. (From Lankester, 'Journ. Linn. Soc.')

Fig. 53.—The same Scorpion carrying the now paralysed fly held in its cheliceræ, the chelæ liberated for attack and defence. Drawn from life. (From Lankester, 'Journ. Linn. Soc.')

young are born fully formed, and are carried by the mother on her back. As many as thirty have been counted in a brood. For information as to the embryology of scorpions the reader is referred to the works named in the bibliography on p. 265. Scorpions do not possess spinning organs, nor form either snares or nests so far as is known; but some species

inhabiting sandy deserts form extensive burrows. The fifth pair of prosomatic appendages is used by these scorpions when burrowing to kick back the sand as the burrow is excavated by the great chelæ.

References to works dealing with the taxonomy and geographical distribution of scorpions are given at the end of this article (**28**).

Section β. Epectinata.—The primitive distinction between the mesosoma and the metasoma wholly or almost wholly obliterated, the two regions uniting to form an opisthosoma, which never consists of more than twelve somites and never bears appendages or breathing organs behind the fourth somite. The breathing organs of the opisthosoma, when present, represented by two pairs of stigmata, opening either upon the first and second (Pedipalpi) or the second and third somites (Solifugæ, pseudo-Scorpiones), or by a single pair upon the third (? second) somite (Opiliones) of the opisthosoma, there being rarely an additional stigma on the fourth (some Solifugæ). The appendages of the second somite of the opisthosoma absent, rarely minute and bud-like (some Amblypygi), never pectiniform. A prægenital somite is often present either in a reduced condition forming a waist (Pedipalpi, Araneæ, Palpigradi) or as a full-sized tergal plate (pseudo-Scorpiones); in some it is entirely atrophied (Solifugæ, Holosomata, and Rhynchostomi). Lateral eyes, when present, diplostichous.

Remarks.—The epectinate Arachnids do not stand so close to the aquatic ancestors of the Embolobranchia as do the pectiniferous scorpions. At the same time we are not justified in supposing that the scorpions stand in any way as an intermediate grade between any of the existing Epectinata and the Delobranchia. It is probable that the Pedipalpi, Araneæ, and Podogona have been separately evolved as distinct lines of descent from the ancient aquatic Arachnida. The Holosomata and Rhynchostomi are probably offshoots from the stem of the Araneæ, and it is not unlikely (in view of the structure of the prosomatic somites of the Tartarides) that

the Solifugæ are connected in origin with the Pedipalpi. The appearance of tracheæ in place of lung-sacs cannot be regarded as a starting-point for a new line of descent comprising all the tracheate forms; tracheæ seem to have developed independently in different lines of descent. On the whole, the Epectinata are highly specialised and degenerate forms, though there are few, if any, animals which surpass the spiders in rapidity of movement, deadliness of attack, and constructive instincts.

Order 2. Pepipalpi (Figs. 54 to 59).—Appendages of first pair bisegmented, without poison gland; of second pair prehensile, their basal segments underlying the proboscis, and furnished with sterno-coxal (maxillary) process, the apical segment tipped with a single movable or immovable claw; appendages of third pair different from the remainder, tactile in function, with at least the apical segment many-jointed and clawless. The ventral surface of the prosoma bears prosternal, metasternal, and usually mesosternal chitin plates (Fig. 55). A narrow prægenital somite is present between opisthosoma and prosoma (Figs. 55, 57). Opisthosoma consisting of eleven somites, almost wholly without visible appendages. Intromittent organ of male beneath the genital operculum (= sternum of the first somite of opisthosoma).

Note.—The possibility of another interpretation of the anterior somites of the mesosoma and the prægenital somite must be borne in mind. Possibly, though not probably, the somites carrying the two lung-sacs correspond to the first two lung-bearing somites of Scorpio, and it is the genital opening which has shifted. The same caution applies in the case of the Araneæ. Excalation of one or of two anterior mesosomatic somites, besides the prægenital somite, would then have to be supposed to have occurred also.

Sub-order *a*. Uropygi.—Prosoma longer than wide, its sternal area very narrow, furnished with a large prosternal and metasternal plate, and often with a small mesosternal sclerite. Appendages of second pair with their basal segments

united in the middle line and incapable of lateral movement; appendages of third pair with only the apical segment many-jointed. Opisthosoma without trace of appendages; its posterior somites narrowed to form a movable tail for the support of the post-anal sclerite, which has no poison glands.

Tribe 1. Urotricha.—Dorsal area of prosoma covered with

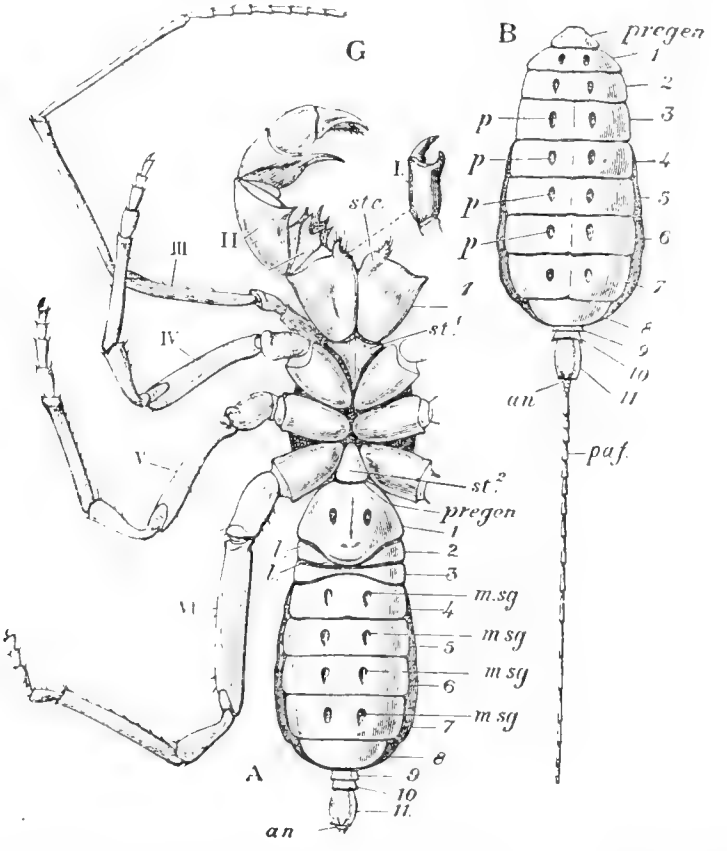


Fig. 54.—Thelyphonus, one of the Pedipalpi. A, ventral view; I, chelicera (detached); II, chelæ; III, palpiform limb; IV to VI, the walking legs; *stc*, sterno-coxal process (gnathobase) of the chelæ; st^1, anterior sternal plate of the prosoma; st^2, posterior sternal plate of the prosoma; *pregen*, position of the præ-genital somite (not seen); *l*, *l*, position of the two pulmonary sacs of the right side; 1 to 11, somites of the opisthosoma (mesosoma plus metasoma); *msg*, stigmata of the tergo-sternal muscles; *an*, anus. B, dorsal view of the opisthosoma of the same; *pregen*, the præ-genital somite; *p*, the tergal stigmata of the tergo-sternal muscles; *paf*, post-anal segmented filament corresponding to the post-anal spine of Limulus. (From Lankester, 'Quart. Journ. Micr. Sci.,' N.S., vol. xxi, 1881.)

a single shield (? two in Geralinura), bearing median and lateral eyes. Post-anal sclerite modified as a long, many-

jointed feeler. Appendages of second pair folding in a horizontal plane, complete chelate, the claw immovably united to the sixth segment. Respiratory organs present in the form of pulmonary sacs.

Family Thelyphonidæ (Thelyphonus [Fig. 54], Hypoctonus, *Geralinura).

Tribe 2. Tartarides.—Small degenerate forms, with the dorsal area of the prosoma furnished with two shields, a larger in front covering the anterior four somites, and a smaller behind covering the fifth and sixth somites; the latter generally subdivided into a right and left portion;

Fig. 55.

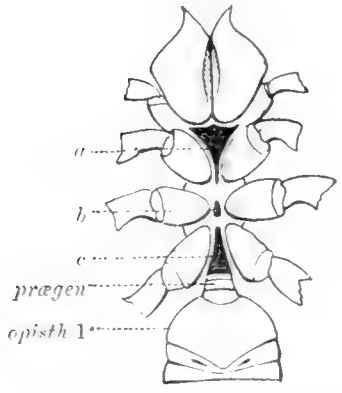


Fig. 56.

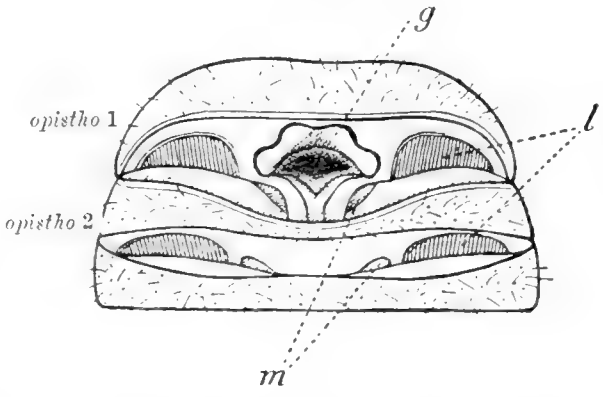


Fig. 55.—Thelyphonus sp. Ventral view of the anterior portion of the body to show the three prososomatic sternal plates *a*, *b*, *c*, and the rudimentary sternal element of the præ-genital somite; *opistho* 1, first somite of the opisthosoma. (From a drawing made by Mr. Pickard-Cambridge, under the directien of Mr. R. I. Pocock.)

Fig. 56.—Thelyphonus assamensis ♂. Ventral surface of the anterior region of the opisthosoma, the first somite being pushed upwards and forwards so as to expose the subjacent structures. *opistho* 1, first somite of the opisthosoma; *opistho* 2, second do.; *g*, genital aperture; *l*, edges of the lamellæ of the lung-books; *m*, stigmata of tergo-sternal muscles. (Original drawing by Mr. Pocock.)

rarely there is a pair of narrow sclerites interposed between the anterior and posterior shields. Eyes evanescent or absent. Appendages of second pair folding in a vertical plane, not chelate, the claw long and movable. Post-anal sclerite short and undivided. No distinct respiratory stigmata behind the sterna of the first and second somites of the opisthosoma.

Family Hubbardiidæ (Schizomus, Hubbardia) (Figs. 57 to 59).

Sub-order *b*. Amblypygi.—Prosoma wider than long, covered above by a single shield bearing median and lateral eyes, which have diplostichous ommatea. Sternal area broad, with prosternal, two mesosternal, and metasternal plates, the prosternum projecting forwards beneath the coxæ of the second pair of appendages. Appendages of second pair folding in a horizontal plane; their basal segments freely movable; claw

Fig. 57.

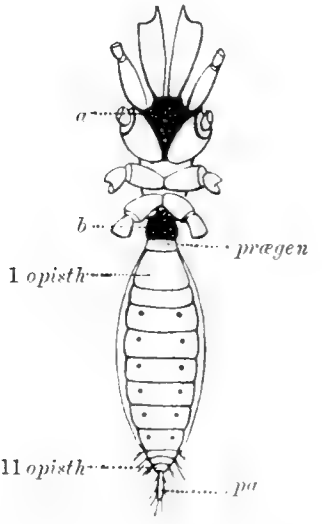


Fig. 58.

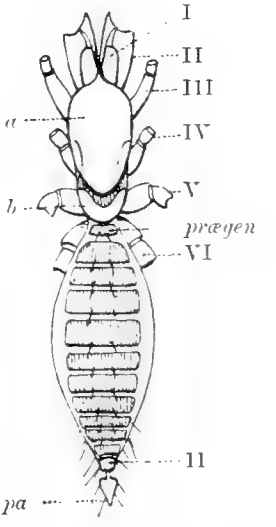


Fig. 57.—Schizomus crassicaudatus, one of the Tartarid Pedipalpi. Ventral view of a female with the appendages cut short near the base. *a*, prosternum of prosoma; *b*, metasternum of prosoma; *prægen*, the præ-genital somite; 1 *opisth*, first somite of the opisthosoma; 11 *opisth*, eleventh somite of the opisthosoma; *pa*, post-anal lobe of the female (compare the jointed filament in Thelyphonus, Fig. 54). (Original drawing by Mr. Pickard-Cambridge, directed by Mr. Pocock).

Fig. 58.—Schizomus crassicaudatus, a Tartarid Pedipalp. Dorsal view of a male with the appendages cut short. I to VI, the prosomatic appendages; *a*, anterior plate, and *b*, posterior plate of the prosomatic carapace; *prægen*, tergum of the prægenital somite; 11, the eleventh somite of the opisthosoma; *pa*, post-anal lobe of the male—a conical body with narrow basal stalk. (Original as above.)

free or fused; basal segments of fourth and fifth pairs widely separated by the sternal area; appendages of third pair with all the segments except the proximal three, forming a many-

jointed flagellum. Opisthosoma without post-anal sclerite and posterior caudal elongation, with frequently a pair of small lobate appendages on the sternum of the third somite. Respiratory organs as in Urotricha.

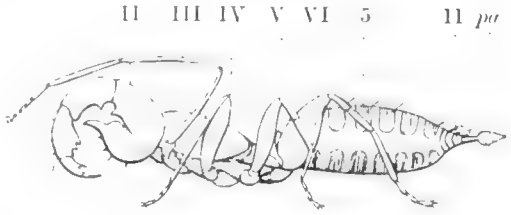


Fig. 59.—Schizomus crassicaudatus, one of the Pedipalpi. Lateral view of a male. II to VI, the prosomatic appendages, the first being concealed (see Fig. 58); 5, the fifth, and 11, the eleventh tergites of the opisthosoma; *pa*, the conical post-anal lobe. (Original as above.)

Family—Phrynichidæ (Phrynichus, Damon).
„ Admetidæ (Admetus Heterophrynus).
„ Charontidæ (Charon, Sarax).
(Family ?) *Geraphrynus.

Remarks.—The Pedipalpi are confined to the tropics and warmer temperate regions of both hemispheres. Fossil forms occur in the Carboniferous. The small forms known as Schizomus and Hubbardia are of special interest from a morphological point of view. The Pedipalpi have no poison glands. (Reference to literature, **29**.)

Order 3. Araneæ (Figs. 60 to 64).—Prosoma covered with a single shield and typically furnished with median and lateral eyes of diplostichous structure, as in the Amblypygi. Its sternal surface wide, continuously chitinised, but with prosternal and metasternal elements generally distinguishable at the anterior and posterior ends respectively of the large mesosternum. Prosternum underlying the proboscis. Appendages of first pair have two segments, as in Pedipalpi, but are furnished with poison gland, and are retroverts. Appendages of second pair not underlying the mouth, but freely movable, and except in primitive forms furnished with a maxillary lobe; the rest of the limb like the legs, tipped

with a single claw, and quite unmodified (except in ♂). Remaining pairs of appendages similar in form and function, each tipped with two or three claws. Opisthosoma, when segmented, showing the same number of somites as in the Pedipalpi; usually unsegmented, the prægenital somite constricted to form the waist; the appendages of its third and fourth somites retained as spinning mammillæ. Respiratory

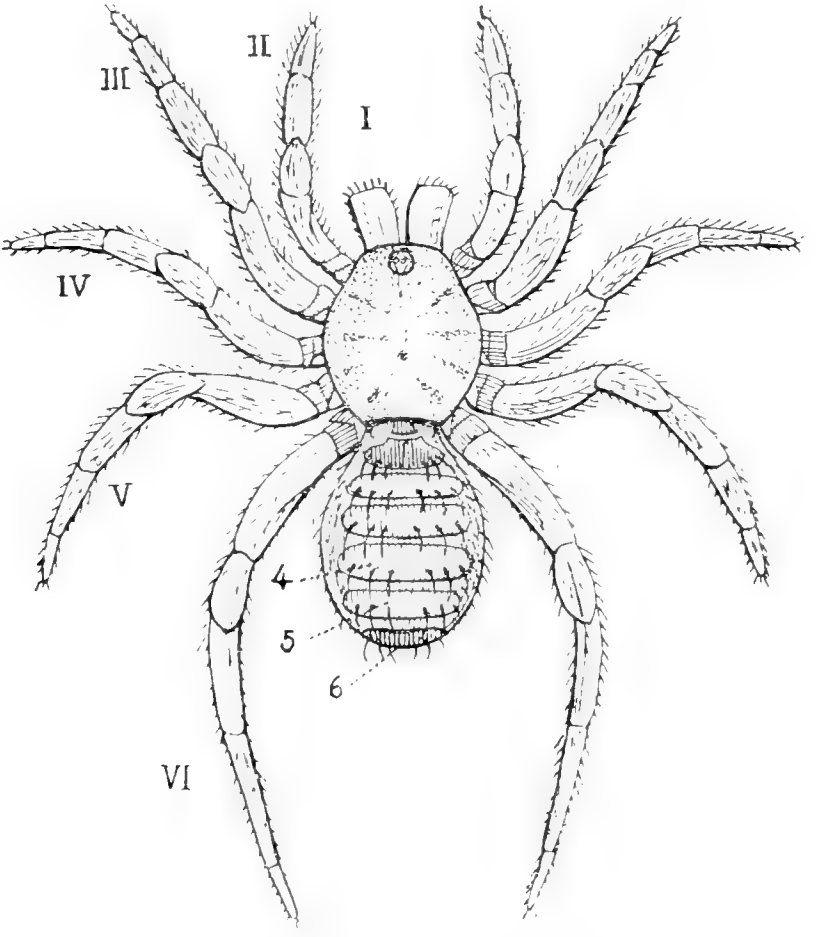


Fig. 60.—Liphistius desultor, Schiödte, one of the Aranеæ Mesothelæ. Dorsal view. I to VI, the prosomatic appendages; 4, 5, 6, the fourth, fifth, and sixth tergites of the opisthosoma. Between the bases of the sixth pair of limbs and behind the prosomatic carapace is seen the tergite of the small prægenital somite. (Original by Pickard-Cambridge and Pocock.)

organs (see Fig. 63, *stg*), as in the Amblypygi, or with the posterior pair, rarely the anterior pair as well, replaced by

tracheal tubes. Intromittent organ of male in the apical segment of the second prosomatic appendage.

Sub-order *a*. Mesothelæ (see Figs. 60—62).—Opisthosoma distinctly segmented, furnished with eleven tergal plates, as in the Amblypygi; the ventral surface of the first and second somites with large sternal plates, covering the genital aperture

Fig. 61.

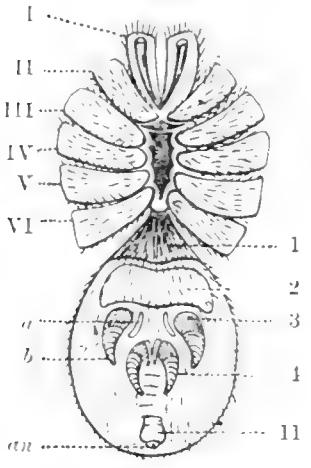


Fig. 62.

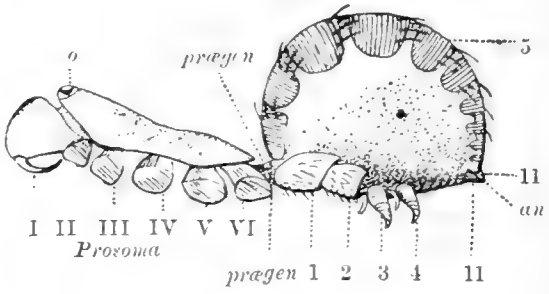


Fig. 61.—Liphistius desultor. Ventral view with the prosomatic appendages cut short excepting the cheliceræ (1) whose sharp retroverts are seen. Between the bases of the prosomatic limbs an anterior and a posterior sternal plate (black) are seen. 1, the sternum of the first opisthosomatic or genital somite covering the genital aperture and the first pair of lung-sacs. In front of it the narrow waist is formed by the soft sternal area of the prægenital somite; 2, the sternite of the second opisthosomatic somite covering the posterior pair of lung-sacs; 3 and 4, the spinning appendages (limbs) of the opisthosoma; *a*, inner, *b*, outer ramus of the appendage; 11, sternite of the eleventh somite of the opisthosoma: in front of it other rudimentary sternites; *an*, anus. (Original as above.)

Fig. 62.—Liphistius desultor. Lateral view. I to VI, appendages of the prosoma cut off at the base; *o*, ocular tubercle; *prægen*, the prægenital somite; 1 and 2, sternites of the first and second opisthosomatic somites; 3 and 4, appendages of the third and fourth opisthosomatic somites, which are the spinning organs, and in this genus occupy their primitive position instead of migrating to the anal region as in other spiders; 5, tergite of the fifth opisthosomatic somite; 11, eleventh opisthosomatic somite; *an*, anus. (Original.)

and the two pairs of pulmonary sacs, the sternal plates from the sixth to the eleventh somites represented by integumental

ridges, weakly chitinised in the middle. The two pairs of spinning appendages retain their primitive position in the middle of the lower surface of the opisthosoma far in advance of the anus on the third and fourth somites, each appendage consisting of a stout, many-jointed outer branch and a slender, unsegmented inner branch. Prosoma as in the Mygalomorphæ, except that the mesosternal area is long and narrow.

Family Liphistiidæ (Liphistius, *Arthrolycosa).

Sub-order *b*. Opisthothelæ (see Fig. 63).—Opisthosoma without trace of separate terga and sterna, the segmentation merely represented posteriorly by slight integumental folds and the sterna of the first and second somites by the opercular plates of the pulmonary sacs. The spinning appendages migrate to the posterior end of the opisthosoma and take up a position close to the anus; the inner branches of the anterior pair either atrophy or are represented homogenetically by a plate, the cribellum, or by an undivided membranous lobe, the colulus.

Tribe 1. Mygalomorphæ.—The plane of the articulation of the appendages of the first pair to the prosoma (the retrovert) vertical, the basal segment projecting straight forwards at its proximal end, the distal segment or fang closing backwards in a direction subparallel to the long axis of the body. Two pairs of pulmonary sacs.

Families: Theraphosidæ (Avicularia, Pœcilotheria). Barychelidæ (Barychelus, Plagiobothrus). Dipluridæ (Diplura, Macrothele). Ctenizidæ (Cteniza, Nemesia). Atypidæ (Atypus, Calommata).

Tribe 2. Arachnomorphæ.—The plane of the articulation of the appendages of the first pair to the prosoma horizontal, the basal segment projecting vertically downwards, at least at its proximal end, the distal segment or fang closing inwards nearly or quite at right angles to the long axis of the body. The posterior pulmonary sacs (except in Hypochilus) replaced by tracheal tubes; the anterior and posterior pairs replaced by tracheal tubes in the Caponiidæ.

Principal families: Hypochilidæ (Hypochilus). Dysderidæ (Dysdera, Segestria). Caponiidæ (Caponia, Nops). Filistatidæ (Filistata). Uloboridæ (Uloborus, Dinopis). Argiapidæ (Nephila, Gasteracantha). Pholcidæ (Pholcus, Artema). Agelenidæ (Tegenaria). Lycosidæ (Lycosa). Clubionidæ (Clubiona, Olios, Sparas-

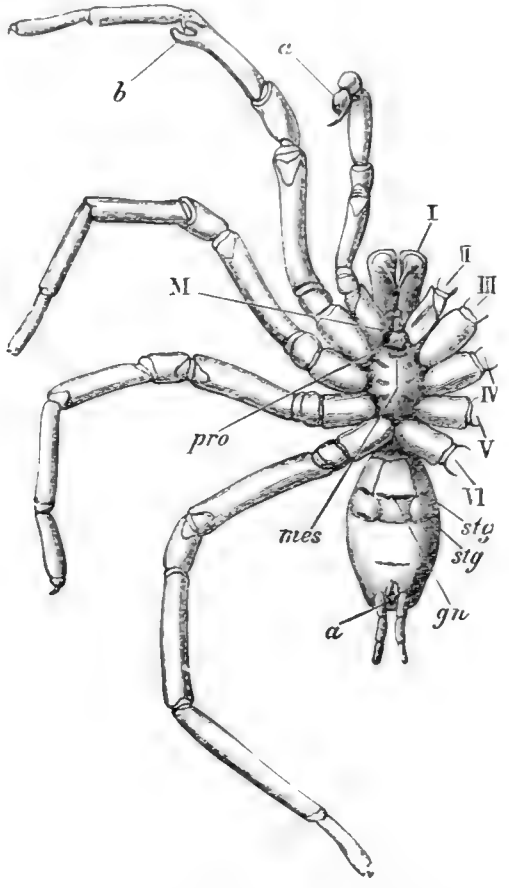


FIG. 63.—Ventral view of a male mygalomorphous Spider. I to VI, the six pairs of prosomatic appendages; *a*, copulatory apparatus of the second appendage; *b*, process of the fifth joint of the third appendage; M, mouth; *pro*, prosternite of the prosoma; *mes*, mesosternite of the prosoma: observe the contact of the coxæ of the sixth pair of limbs behind it; compare Liphistius (Fig. 61) where this does not occur; *stg*, lung aperture; *gn*, genital aperture; *a*, anus with a pair of backwardly migrated spinning appendages on each side of it; compare the position of these appendages in Liphistius (Fig. 61). (From Lankester, "Limulus an Arachnid.")

sus). Gnaphosidæ (Gnaphosa, Hemiclæa). Thomisidæ (Thomisus). Attidæ (Salticus). Urocteidæ (Uroctea). Eresidæ (Eresus).

Remarks on the Araneæ.—The spiders are the most numerous and diversified group of the Arachnida; about 2000 species are known. No noteworthy fossil spiders are known; the best preserved are in amber of Oligocene age. Protolycosa and Arthrolycosa occur in the Carboniferous. Morphologically the spiders are remarkable for the concentration and specialisation of their structure, which is accompanied with high physiological efficiency. The larger

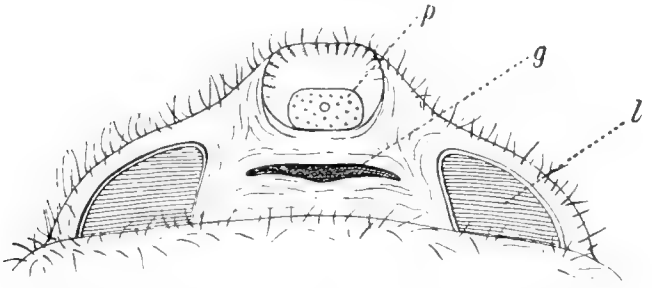


Fig. 64.—Liphistius desultor. Under side of the uplifted genital or first opisthosomatic somite of the female; *g*, genital aperture; *p*, pitted plate, probably a gland for the secretion of adhesive material for the eggs; *l*, the edges of the lamellæ of the lung-books of the first pair. (Original drawing by Pocock.)

species of Bird's-nest Spiders (Avicularia), the opisthosoma of which is as large as a bantam's egg, undoubtedly attack young birds, and M'Cook gives an account of the capture in its web by an ordinary house spider of a small mouse. The "retrovert" or bent-back first pair of appendages is provided with a poison gland opening on the fang or terminal segment. Spiders form at least two kinds of construction—snares for the capture of prey and nests for the preservation of the young. The latter are only formed by the female, which is a larger and more powerful animal than the male. Like the scorpions the spiders have a special tendency to cannibalism, and accordingly the male, in approaching the female for the purpose of fertilising her, is liable to be fallen upon and sucked dry by the object of his attentions. The sperm is removed by the male from the genital aperture into a special receptacle on the terminal segment of the second prosomatic appendage. Thus held out at some distance from the body,

it is cautiously advanced by the male spider to the genital aperture of the female.

For an account of the courtship and dancing of spiders, of their webs and floating lines, the reader is referred to the

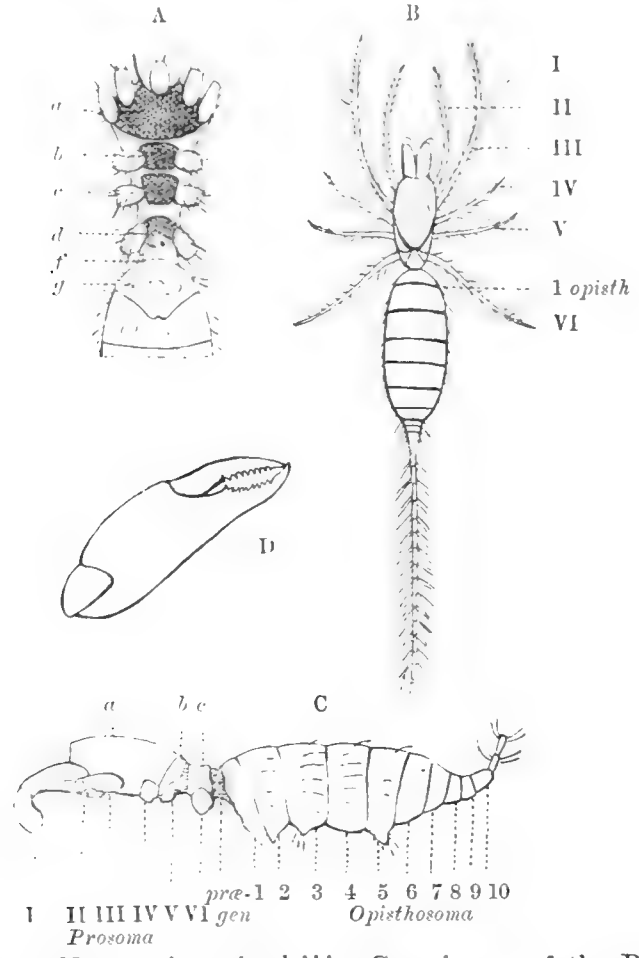


FIG. 65.—Kœnenia mirabilis, Grassi, one of the Palpigradi. A, ventral view of prosoma and of anterior region of opisthosoma with the appendages cut off near the base; *a* and *b*, prosternites; *c*, mesosternite; and *d*, metasternite of the prosoma; *f*, ventral surface of the prægenital somite; *g*, sternite of the genital somite (first opisthosomatic somite). B, dorsal view. I to VI, prosomatic appendages; 1 *opisth*, genital somite (first opisthosomatic somite). C, lateral view. I to VI, prosomatic appendages; *a*, *b*, *c*, the three tergal plates of the prosoma; *prægen*, the prægenital somite; 1 to 10, the ten somites of the opisthosoma. D, chelicera. (Original drawing by Pocock and Pickard-Cambridge, after Hansen and Sörensen.)

works of M'Cook (**30**) and the Peckhams (**31**), whilst an excellent account of the nests of trap-door spiders is given by

Moggridge (**32**). References to systematic works will also be found at the end of this article (**33**).

Order 4. Palpigradi = Microthelyphonida (see Fig. 65).—Prosoma covered above by three plates, a larger representing the dorsal elements of the first four somites, and two smaller representing the dorsal elements of the fifth and sixth.

Its ventral surface provided with one prosternal, two mesosternal, and one metasternal plate. Appendages of first pair consisting of three segments, completely chelate, without poison gland; of second pair slender, leg-like, tipped with three claws, the basal segment without sterno-coxal process, taking no share in mastication, and widely separated from its fellow of the opposite side; third, fourth, fifth, and sixth appendages similar in form to the second and to each other.

Proboscis free, not supported from below by either the prosternum or the basal segments of the appendages of the second pair.

Opisthosoma consisting of only ten somites, which have no tergal and sternal elements, the prægenital somite contracted to form a "waist," as in the Pedipalpi; the last three narrowed to form a caudal support for the many-jointed flagelliform telson, as in the Urotricha. Respiratory organs atrophied.

Family Kœneniidæ (Kœnenia).

Remarks.—An extremely remarkable minute form originally described by Grassi (**34**) from Sicily, and since further described by Hansen (**35**). Recently the genus has been found in Texas, U.S.A. Only one genus of the order is known.

Order 5. Solifugæ = Mycetophoræ (see Figs. 66—69).—Dorsal area of prosoma covered with three distinct plates, two smaller representing the terga of the fifth and sixth somites, and a larger representing those of the anterior four somites, although the reduced terga of the third and fourth are traceable behind the larger plate. The latter bears a pair of median eyes and obsolete lateral eyes on each side. Sternal elements of prosoma almost entirely absent, traces of a

prosternum and metasternum alone remaining. Rostrum free, not supported by either the prosternum or the basal segments of the appendages. Appendages of first pair large, chelate, bisegmented, articulated to the sides of the head-shield; appendages of second pair simple, pediform, with protrusible (? suctorial) organ, and no claws at the tip; their basal segments united in the middle line and furnished with sterno-coxal process. Remaining pairs of appendages with

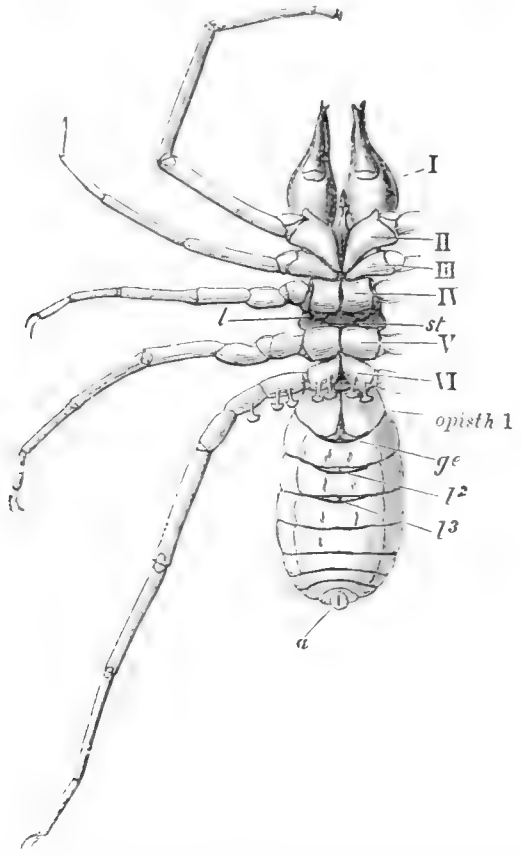


FIG. 66.—Galeodes, sp., one of the Solifugæ. Ventral view to show legs and somites. I to VI, the six leg-bearing somites of the prosoma; *opisth* 1, first or genital somite of the opisthosoma; *ge*, site of the genital aperture; *st*, thoracic tracheal aperture; l^2, anterior tracheal aperture of the opisthosoma in somite 2 of the opisthosoma; l^3, tracheal aperture in somite 3 of the opisthosoma; *a*, anus. (From Lankester, "Limulus an Arachnid.")

their basal segments immovably fixed to the sternal surface, similar in form, the posterior three pairs furnished with two claws supported on long stalks; the basal segments of the

sixth pair bearing five pairs of tactile sensory organs or malleoli. The prægenital somite is suppressed. Opisthosoma composed of ten somites. Respiratory organs tracheal, open-

Fig. 67.

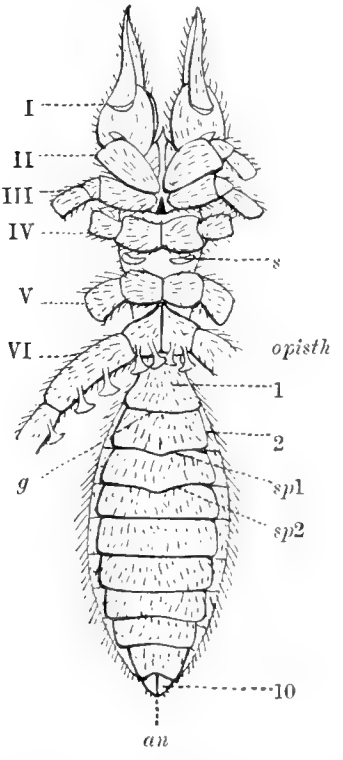


Fig. 68.

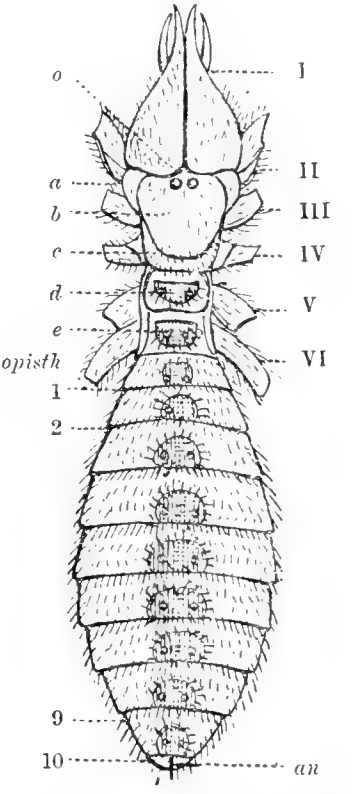


Fig. 67.—Galeodes, sp., one of the Solifugæ. Ventral view with the appendages cut off at the base. I to VI, prosomatic appendages; *s*, prosomatic stigma or aperture of the tracheal system; 1, first opisthosomatic sternite covering the genital aperture *g*; 2, second opisthosomatic sternite covering the second pair of tracheal apertures *sp*1; *sp*2, the third pair of tracheal apertures; 10, the tenth opisthosomatic somite; *an*, the anal aperture. (Original by Pickard-Cambridge and Pocock.)

Fig. 68.—Galeodes, sp., one of the Solifugæ. Dorsal view. I to VI, bases of the prosomatic appendages; *o*, eyes; *a*, lateral region of the cephalic plate to which the first pair of appendages are articulated; *b*, cephalic plate with median eye; *c*, dorsal element of somites bearing third and fourth pairs of appendages; *d*, second plate of the prosoma with fifth pair of appendages; *e*, third or hindermost plate of the prosoma beneath which the sixth pair of legs is articulated; 1, 2, 9, 10, first, second, ninth, and tenth somites of the opisthosoma; *an*, anus. (Original.)

ing upon the ventral surface of the second and third, and sometimes also of the fourth somite of the opisthosoma. A

supplementary pair of tracheæ opening behind the basal segment of the fourth appendage of the prosoma.

Intromittent organ of male lodged on the dorsal side of the first pair of prosomatic appendages.

Families: Hexisopodidæ (Hexisopus). Solpugidæ (Solpuga, Rhagodes). Galeodidæ (Galeodes).

Remarks.—These most strange-looking Arachnids occur in warmer temperate, and tropical regions of Asia, Africa, and America. Their anatomy has not been studied as yet by means of freshly killed material, and is imperfectly known, though the presence of the coxal glands was determined by Macleod in 1884. The proportionately enormous chelæ

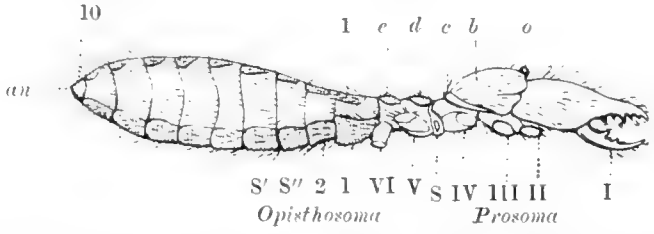


FIG. 69.—Galeodes, sp., one of the Solifugæ. I to VI, the six prosomatic limbs cut short; *o*, the eyes; *b*, *c*, demarcated areæ of the cephalic or first prosomatic plate corresponding respectively to appendages I, II, III and to appendage IV (see Fig. 68); *d*, second plate of the prosoma-carrying appendage V; *e*, third plate of the prosoma-carrying appendage VI. The prægenital somite is absent. 1, first somite of the opisthosoma; 2, second do.; S, prosomatic tracheal aperture between legs IV and V; S' and S'', opisthosomatic tracheal apertures; 10, tenth opisthosomatic somite; *an*, anus. (Original.)

(cheliceræ) of the first pair of appendages are not provided with poison glands; their bite is not venomous.

Galeodes has been made the means of a comparison between the structure of the Arachnida and Hexapod insects by Haeckel and other writers, and it was at one time suggested that there was a genetic affinity between the two groups—through Galeodes, or extinct forms similar to it. The segmentation of the prosoma and the form of the appendages bear a homoplastic similarity to the head, pro-, meso-, and meta-thorax of a Hexapod with mandibles, maxillary palps, and three pairs of walking legs; whilst the

opisthosoma agrees in form and number of somites with the abdomen of a Hexapod, and the tracheal stigmata present certain agreements in the two cases. (Reference to literature, **36.**)

Order 6. Pseudoscorpiones = Chelonethi, also called Chernetidia (see Figs. 70—72).—Prosoma covered by a single dorsal shield, at most furnished with one or two diplostichous

Fig. 70.

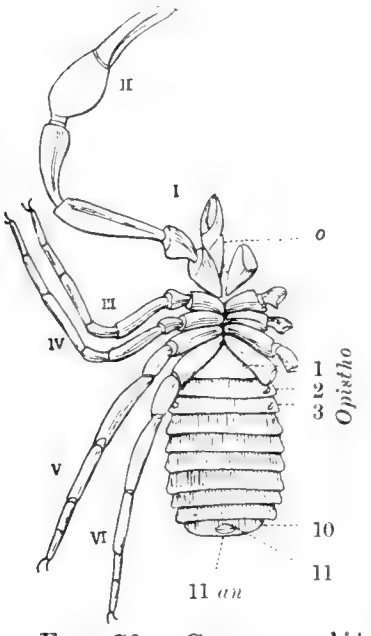


Fig. 71.

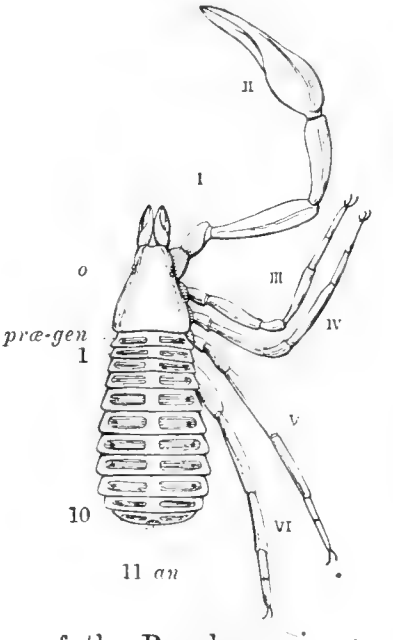


Fig. 70.—Garypus litoralis, one of the Pseudoscorpiones. Ventral view. I to VI, prosomatic appendages; *o*, sterno-coxal process of the basal segment of the second appendage; 1, sternite of the genital or first opisthosomatic somite; the prægenital somite, though represented by a tergum, has no separate sternal plate; 2 and 3, sternites of the second and third somites of the opisthosoma, each showing a tracheal stigma; 10 and 11, sternites of the tenth and eleventh somites of the opisthosoma; *an*, anus. (Original by Pocock and Pickard-Cambridge.)

Fig. 71.—Garypus litoralis, one of the Pseudoscorpiones. Dorsal view. I to VI, the prosomatic appendages; *o*, eyes; *prægen*, prægenital somite; 1 tergite of the genital or first opisthosomatic somite; 10, tergite of the tenth somite of the opisthosoma; 11, the evanescent eleventh somite of the opisthosoma; *an*, anus. (Original.)

lateral eyes; sternal elements obliterated or almost obliterated. Appendages of the first pair bisegmented completely chelate, furnished with peculiar organs, the serrula

and the lamina. Appendages of second pair very large and completely chelate, their basal segments meeting in the middle line, as in the Uropygi, and provided in front with membranous lip-like processes underlying the proboscis. Appendages of the third, fourth, fifth, and sixth pairs similar in form and function, tipped with two claws, their basal segments in contact in the median ventral line. The præ-genital somite wide, not constricted, with large tergal plate, but with its sternal plate small or inconspicuous. Opisthosoma composed, at least in many cases, of eleven somites, the eleventh somite very small, often hidden within the tenth. Respiratory organs in the form of tracheal tubes opening by a pair of stigmata in the second and third somites of the opisthosoma. Intromittent organ of male beneath sternum of the first somite of the opisthosoma.

Sub-order *a*. Panctenodactyli.—Dorsal plate of prosoma (carapace) narrowed in front; the appendages of the first pair small, much narrower, taken together, than the posterior border of the carapace. Serrula on movable digit of appendages of first pair fixed throughout its length, and broader at its proximal than at its distal end; the immovable digit with an external process.

Family Cheliferidæ (Chelifer (Figs. 66, 67, 68), Chiridium).

„ Garypidæ (Garypus).

Sub-order *b*. Hemictenodactyli.—Dorsal plate of prosoma scarcely narrowed in front; the appendages of the first pair large, not much narrower, taken together, than the posterior border of the carapace. The serrula or the movable digit free at its distal end, narrowed at the base; no external lamina on the immovable digit.

Family Obisiidæ (Obisium, Pseudobisium).

„ Chthoniidæ (Chthonius, Tridenchthonius).

Remarks.—The book-scorpions—so called because they were, in old times, found not unfrequently in libraries—are found in rotten wood and under stones. The similarity of the form of their appendages to those of the scorpions

suggests that they are a degenerate group derived from the latter, but the large size of the prægenital somite in them

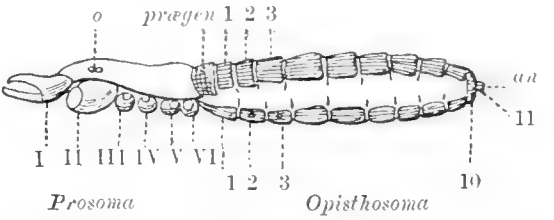


FIG. 72.—Garypus litoralis, one of the Pseudoscorpiones. Lateral view. I to VI, basal segments of the sixth prosomatic appendages; *o*, eyes; *prægen*, tergite of the prægenital somite; 1, genital or first opisthosomatic somite; 2, 3, 10, the second, third, and tenth somites of the opisthosoma; 11, the minute eleventh somite; *an*, the anus. (Original.)

would indicate a connection with forms preceding the scorpions. (Reference to literature, **37.**)

Order 7. Podogona = Meridogastra (see Figs. 73 to 76).—

FIG. 73.

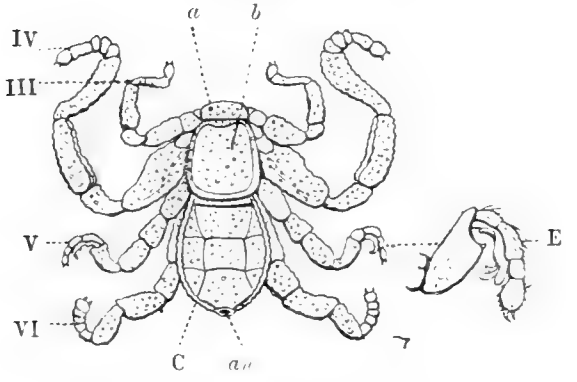


FIG. 74.

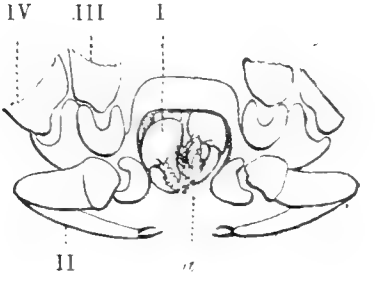


FIG. 73.—Cryptostemma Karschii, one of the Podogona. Dorsal view of male, enlarged five times linear. III to VI, the third, fourth, fifth, and sixth appendages of the prosoma; *a*, movable (hinged) sclerite (so-called hood) overhanging the first pair of appendages; *b*, fused terga of the prosoma followed by the opisthosoma of four somites; *an*, anus; E, extremity of the fifth appendage of the male modified to subserve copulation. (Original drawing by Pocock and Pickard-Cambridge.)

FIG. 74.—Cryptostemma Karschii. Anterior aspect of the prosoma with the "hood" removed. I to IV, first to fourth appendages of the prosoma; *a*, basal segment of the second pair of appendages meeting its fellow in the middle line (see Fig. 75). (Original drawing by Pocock and Pickard-Cambridge.)

Dorsal area of prosoma furnished with two shields, a larger behind representing, probably, the tergal elements of the

somites, and a smaller in front, which is freely articulated to the former and folds over the appendages of the first pair. Ventral area without distinct sternal plates. Appendages of first pair bi-segmented, completely chelate. Appendages of second pair with their basal segments uniting in the middle line below the mouth, weakly chelate at apex. Appendages of third, fourth, fifth, and sixth pairs similar in form; their basal segments in contact in the middle line

Fig. 75.

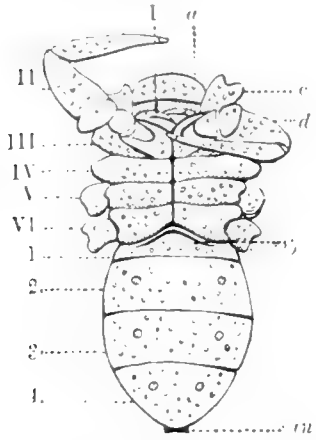


Fig. 76.

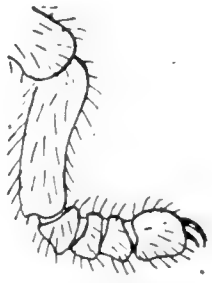


Fig. 75.—Cryptostemma Karschii, one of the Podogona. Ventral view. I to VI, the six pairs of appendages of the prosoma, the last three cut short; 1, 2, 3, 4, the four somites of the opisthosoma; *a*, hood overhanging the first pair of appendages; *b*, position of the genital orifice; *c*, part of third appendage; *d*, fourth segment of second appendage. Observe that the basal segment of appendage III does not meet its fellow in the middle line. (Original drawing by Pocock and Pickard-Cambridge.)

Fig. 76.—Cryptostemma Karschii. Extremity of the fifth pair of appendages of the female for comparison with that of the male E in Fig. 73.

and immovably welded, except those of the third pair, which have been pushed aside so that the bases of the second and fourth pairs are in contact with each other. A movable membranous joint between the prosoma and the opisthosoma, the generative aperture opening upon the ventral side of the membrane. Prægenital somite suppressed, the opisthosoma consisting of only four visible somites, in addition to a tubular ring round the anal orifice. Respiratory organs

unknown. Intromittent organ of male placed at the distal end of the appendage of the fifth pair.

Family Cryptostemmidæ (Cryptostemma) (*Poliochæra), Carboniferous.

Remarks on the Podogona.—The name given to this small but remarkable group has reference to the position of the male intromittent organ (Fig. 73, E). They are small degenerate animals with a relatively firm integument. Not more than four species and twice that number of specimens are known. They have been found in West Africa and South America. A fact of special interest in regard to them is that the genus Poliochæra, from the Coal Measures, appears to be a member of the same group. The name Cryptostemma, given to the first-known genus of the order, described by Guérin-Ménéville, refers to the supposed concealment of the eyes by the movable cephalic sclerite. (Reference to literature, **38**.)

Order 8. Opiliones.[1]—Carapace of prosoma consisting of a short posterior and a large anterior plate, which bears a pair of median or one or two pairs of lateral eyes. Sternal elements consisting of an anterior prosternal sclerite or labium and a posterior metasternal sclerite. Appendages of first pair large, three-jointed, and chelate; of second pair either simple and palpiform or raptorial and subchelate; of remaining pairs similar in form and ambulatory in function; the basal segments of the second, third, and sometimes of the fourth pairs of appendages furnished with sterno-coxal (maxillary) lobe. Opisthosoma confluent throughout its width with the prosoma, consisting sometimes of as many as ten segments, the generative aperture lying far forwards between the basal segments of the sixth or fifth and sixth prosomatic appendages. Prægenital somite suppressed. Respiratory organs, tracheal, a single pair of spiracles opening upon the sternum immediately behind the basal segments of the appendages of the sixth pair; supplementary spiracles sometimes present upon the fifth segment of the legs. Both

[1] Mr. Pocock has furnished me with the above account of the Opiliones to take the place of that which appeared in the Encyclopædia.—E. R. L.

male and female furnished with a large protrusible copulatory organ lying within the generative orifice.

Sub-order *a*. Cyphophthalmi (= Anepignathi).—First sternal plate of the opisthosoma small, not covering the genital aperture, which in the adult forms a gaping orifice. Opisthosoma presenting ten tergal and nine sternal plates. Carapace narrowed anteriorly and produced forwards so as to overlap considerably the basal segment of the appendages

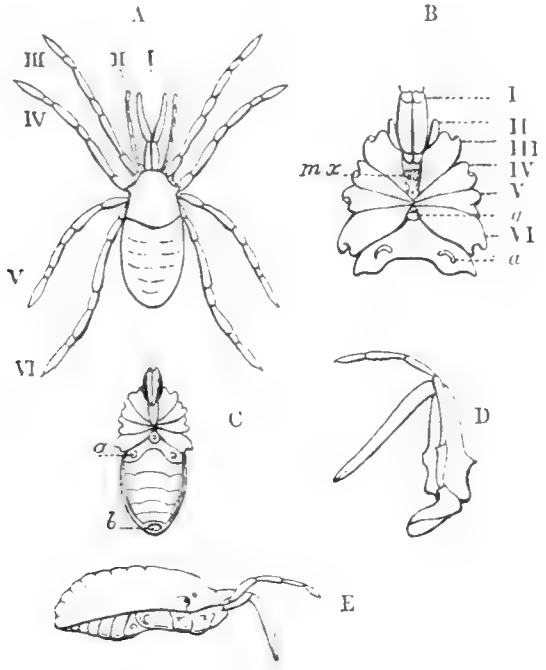


FIG. 77.—Stylocellus sumatranus, one of the Opiliones; after Thorell. Enlarged. A, dorsal view; I to VI, the six prosomatic appendages. B, ventral view of the prosoma and of the first somite of the opisthosoma, with the appendages I to VI cut off at the base; *a*, tracheal stigma; *mx*, maxillary process of the coxæ of the third pair of appendages; *g*, genital aperture. C, ventral surface of the prosoma and opisthosoma; *a*, tracheal stigma; b, last somite. D, lateral view of the first and second pair of appendages. E, lateral view of the whole body and two first appendages, showing the fusion of the dorsal elements of the prosoma into a single plate, and of those of the opisthosoma into an imperfectly segmented plate continuous with that of the prosoma.

of the first pair; basal segments of appendages of second pair meeting in the middle line above the camarostome. Remaining appendages stout, having one claw; their basal

segments immovably fused. Sternum of prosoma almost obliterated.

Family Sironidæ (Siro, Pettalus, Stylocellus).

Sub-order *b*. Mecostethi.—Generative aperture covered by the first sternal plate of the opisthosoma. This region with nine tergal and eight sternal plates. Carapace not produced on each side of the appendages of the first pair. Appendages of second pair raptorial, stout, usually spined, their basal segments not meeting above the camarostome. Coxa of appendages of third pair furnished with immovable maxillary lobe; coxæ of remaining pairs immovable. Metasternal plate long, only its posterior extremity overlapped to a small extent by the sternal plate forming the genital operculum.

Tribe *a*. Laniatores.—With two claws upon the appendages of the fifth and sixth pairs.

Principal families: Cosmetidæ (Cosmetus).
Gonyleptidæ (Gonyleptis).
Assamiidæ (Assamia).
Phalangodidæ (Phalangodes).
Oncopodidæ (Oncopus).

Tribe β. Insidiatores.—With a single bidentate claw upon the appendages of the fifth and sixth pairs.

Principal families: Adæidæ (Adæum, Larifuga).
Triænobunidæ (Triænobunus).
Triænonychidæ (Triænonyx, Acumontia).

Sub-order *c*. Plagiostethi.—Differing from the Mecostethi principally in the fact that the sternal area of the first segment of the opisthosoma is prolonged forwards so as to cover almost entirely the metasternal plate of the prosoma, from which the coxæ of the appendages diverge radially, and in that the appendages of the second pair are weak and unspined.

Tribe *a*. Eupagosterni.—Metasternum of prosoma longitudinal, immovably wedged between the coxæ; prosternum narrow and longitudinal. No distinct maxillary lobe on the coxa of the fourth appendage.

Principal families: Nemastomidæ (Nemastoma).
Trogulidæ (Trogulus).

Tribe β. Apagosterni.—Metasternum of prosoma short, transverse, not immovably wedged between the coxæ; prosternum large, quadrate. A distinct maxillary lobe on the coxa of the fourth appendage.

Families: Ischyropsalidæ (Ischyropsalis).
Phalangiidæ (Phalangium).

Nearly related to the Opiliones are the genera from the Carboniferous strata constituting the group Anthracomarti. These genera, of which the best known are Eophrynus and Anthracomartus, seem to have differed from the existing Opiliones in retaining a movable joint between the prosoma and opisthosoma, and in the presence of movable lateral plates upon the terga of the opisthosoma.

Remarks on the Opiliones.—These include the harvestmen, sometimes also called Daddy-long-legs, with round undivided bodies and very long, easily detached legs. The intromittent organs of the male are remarkable for their complexity and elaboration. The confluence of the regions of the body and the dislocation of apertures from their typical position are results of degeneration. The Opiliones seem to lead on from the spiders to the mites. (Reference to literature, **39**.)

Order 9. Rhynchostomi = Acari (see Fig. 78).—Degenerate Arachnids resembling the Opiliones in many structural points, but chiefly distinguishable from them by the following features:—The basal segments of the appendages of the second pair are united in the middle line behind the mouth; those of the third, fourth, fifth, and sixth pairs are widely separated and not provided with sterno-coxal (maxillary) lobes, and take no share in mastication; the respiratory stigmata, when present, usually belong to the prosoma, and the primitive segmentation of the opisthosoma has entirely or almost entirely disappeared.

Sub-order *a*. Notostigmata.—Opisthosoma consisting of ten segments defined by integumented grooves, the anterior

four of these furnished with a single pair of dorsally-placed spiracles or tracheal stigmata.

Family Opilioacaridæ (Opilioacarus).

Sub-order *b*. Cryptostigmata. — Integument hard,

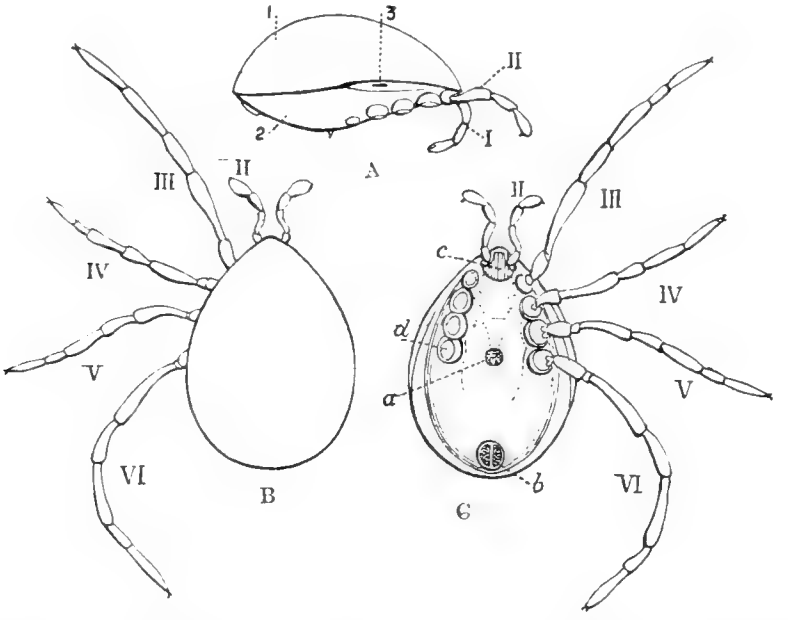


FIG. 78.—Holothyrus nitidissimus, one of the Acari; after Thorell. Enlarged ten times linear. A, lateral view with appendages III to VI removed; 1, plate covering the whole dorsal area, representing the fused tergal sclerites of the prosoma and opisthosoma; 2, similarly formed ventral plate; 3, tracheal stigma. B, dorsal view of the same animal; II to VI, second to sixth pairs of appendages. The first pair of appendages, both in this and in C, are retracted. C, ventral view of the same; II to VI as in B; *a*, genital orifice; *b*, anus; *c*, united basal segments of the second pair of appendages; *d*, basal segment of the sixth prosomatic appendage of the right side. The rest of the appendage, as also of appendages III, IV, and V, has been cut away. (Original drawing by Pocock and Pickard-Cambridge.)

strengthened by a continuously chitinised dorsal and ventral sclerite. Tracheæ typically opening by stigmata situated in the articular sockets (acetabula) of the third, fourth, fifth and sixth pairs of appendages.

Family Oribatidæ (Oribata, Nothrus, Hoplophora).

Sub-order *c*. Metastigmata.—Integument mostly like that

of the Cryptostigmata. Tracheæ opening by a pair of stigmata situated above and behind the base of the fourth or fifth or sixth pair of appendages.

Families: Gamasidæ (Gamasus, Pteroptus).
Argasidæ (Argas, Ornithodoros).
Ixodidæ (Ixodes, Rhipicephalus).

Sub-order *d*. Prostigmata.—Integument soft, strengthened by special sclerites, those on the ventral surface of the prosoma apparently representing the basal segments of the legs imbedded in the skin. Tracheæ, except in the aquatic species in which they are atrophied, opening by a pair of stigmata situated close to or above the base of the appendages of the first pair (cheliceræ).

Families: Trombidiidæ (Trombidium, Tetranychus).
Hydrachnidæ (Hydrachna, Atax).
Halacaridæ (Halacarus, Leptognathus).
Bdellidæ (Bdella, Eupodes).

Sub-order *e*. Astigmata.—Degenerate, mostly parasitic forms approaching the Prostigmata in the development of integumental sclerites and the softness of the skin, but with the respiratory system absent.

Families: Tyroglyphidæ (Tyroglyphus, Rhizoglyphus).
Sarcoptidæ (Sarcoptes, Analges).

Sub-order *f*. Vermiformia.—Degenerate atracheate parasitic forms with the body produced posteriorly into an annulated caudal prolongation, and with the third, fourth, fifth, and sixth pairs of appendages short and only three-jointed.

Family Demodicidæ (Demodex).

Sub-order *g*. Tetrapoda.—Degenerate atracheate gall-mites, in which the body is produced posteriorly and annulated, as in Demodex, but in which the appendages of the third and fourth pairs are long and normally segmented, and those of the fifth and sixth pairs entirely absent.

Family Eriophyidæ (Eriophyes, Phyllocoptes).

Remarks on the Rhynchostomi.—The Acari include a number of forms which are of importance and special interest on account of their parasitic habits. The ticks

(Ixodes) are not only injurious as blood-suckers, but are now credited with carrying the germs of Texan cattle fever, just as mosquitoes carry those of malaria. The itch insect (Sarcoptes scabiei) is a well-known human parasite, so minute that it was not discovered until the end of the eighteenth century, and "the itch" was treated medicinally as a rash. The female burrows in the epidermis much as the female trapdoor spider burrows in turf, in order to make a nest in which to rear her young. The male does not burrow, but wanders freely on the surface of the skin. Demodex folliculorum is also a common parasite of the sebaceous glands of the skin of the face in man, and is frequent in the skin of the dog. Many Acari are parasitic on marine and fresh-water molluscs, and others are found on the feathers of birds and the hairs of mammals. Others have a special faculty of consuming dry, powdery vegetable and animal refuse, and are liable to multiply in manufactured products of this nature, such as mouldy cheese. A species of Acarus is recorded as infesting a store of powdered strychnine, and feeding on that drug, so poisonous to larger organisms. (Reference to literature, **40**.)

Authorities cited by numbers in the text.

1. Straus-Durkheim (as reported by MM. Riester and Sanson in an appendix to the sixth volume of the French translation of Meckel's 'Anatomy,' 1829).
2. Lankester.—"Limulus an Arachnid," 'Quart. Journ. Micr. Sci.,' vol. xxi, N.S., 1881.
3. Lankester.—"On the Skeletotrophic Tissues of Limulus, Scorpio, and Mygale," 'Quart. Journ. Micr. Sci.,' vol. xxiv, N.S., 1884.
4. Lankester.—'Trans. Zool. Soc.,' vol. xi, 1883.
5. Lankester and Bourne.—"Eyes of Limulus and Scorpio," 'Quart. Journ. Micr. Sci.,' vol. xxiii, N.S., Jan., 1883.
6. Milne-Edwards, A.—"Recherches sur l'anatomie des Limules," 'Ann. Sci. Nat.,' 5th series, "Zoologie," vol. xvii, 1873.
7. Owen, Richard.—"Anatomy of the King-crab," 'Trans. Linn. Soc. Lond.,' vol. xxviii, 1872.

8. Kishinouye.—"Development of Limulus longispina," 'Journal of the Science College of Japan,' vol. v, 1892.

9. Brauer.—"Development of Scorpion," 'Zeitschrift für wiss. Zoologie,' vol. lix, 1895.

10. Hansen.—"Organs and Characters in Different Orders of Arachnida," 'Entomol. Meddel.,' vol. iv, pp. 137—149.

11. Watase.—"On the Morphology of the Compound Eyes of Arthropods," 'Studies from the Biolog. Lab. Johns Hopkins University,' vol. iv, pp. 287—334.

12. Newport, George.—"Nervous and Circulatory Systems in Myriapoda and Macrourous Arachnids," 'Phil. Trans. Roy. Soc.,' 1843.

13. Lankester.—"Coxal Glands of Limulus, Scorpio, and Mygale," 'Quart. Journ. Micr. Sci.,' vol. xxiv, N.S., 1884.

13A. Patten and Hazen.—"Development of the Coxal Glands of Limulus," 'Journ. of Morphology,' vol. xvi, 1900.

13B. Bernard.—"Coxal Glands of Scorpio," 'Ann. and Mag. Nat. Hist.,' vol. xii, 1893, p. 55.

14. Benham.—"Testis of Limulus," 'Trans. Linn. Soc.,' 1882.

15. Lankester.—"Mobility of the Spermatozoa of Limulus," 'Quart. Journ. Micr. Sci.,' vol. xviii, N.S., 1878.

16. Korschelt and Heider.—'Entwickelungsgeschichte,' Jena, 1892. Ibique citata.

17. Laurie.—"The Embryology of a Scorpion," 'Quart. Journ. Micr. Sci.,' vol. xxxi, N.S., 1890; and "On Development of Scorpio fulvipes," ibid., vol. xxxii, 1891.

18. Lankester.—(Homoplasy and Homogeny.) "On the Use of the Term Homology in Modern Zoology," 'Ann. and Mag. Nat. Hist.,' 1870.

19. Lankester.—"Degeneration: a Chapter in Darwinism," 1878, reprinted in the 'Advancement of Science,' Macmillan, 1890.

20. Lankester.—"Limulus an Arachnid," 'Quart. Journ. Micr. Sci.,' vol. xxi, N.S.

21. Claus.—"Degeneration of the Acari and Classification of Arthropoda," 'Anzeiger d. k. k. Akad. Wissen. Wien,' 1885; see also 'Ann. and Mag. Nat. Hist.' (5), vol. xvii, 1886, p. 364, and vol. xix, p. 225.

22. Lindström, G.—"Researches on the Visual Organs of the Trilobites," 'K. Svenska Vet. Akad. Handl.,' xxxiv, No. 8, pp. 1—86, pls. i—vi, 1901.

22*. Zittel.—American edition of his 'Palæontology' (the Macmillan Co., New York), where ample references to the literature of Trilobitæ and Eurypterini will be found; also references to literature of fossil scorpions and spiders.

23. Hoek.—"Report on the Pycnogonida," 'Challenger Expedition Reports,' 1881. Meinert.—"Pycnogonida of the Danish Ingolf Expedition," vol. iii, 1899. Morgan.—"Embryology and Phylogeny of the Pycnogonids," 'Biol. Lab. Baltimore,' vol. v, 1891.

24. Bourne, A. G.—"The Reputed Suicide of the Scorpion," 'Proc. Roy. Soc.,' vol. xlii, pp. 17—22.

25. Lankester.—"Notes on some Habits of Scorpions," 'Journ. Linn. Soc. Zool.,' vol. xvi, p. 455, 1882.

26. Huxley.—"Pharynx of Scorpion," 'Quart. Journ. Micr. Sci.,' vol. viii (old series), 1860, p. 250.

27. Pocock.—"How and why Scorpions hiss," 'Natural Science,' vol. ix, 1896. Cf. idem, "Stridulating Organs of Spiders," 'Ann. and Mag. Nat. Hist.' (6), xvi, pp. 230—233.

28. Kraepelin.—'Das Thierreich (Scorpiones et Pedipalpi),' Berlin, 1899. Peters.—"Eine neue Eintheilung der Skorpione," 'Mon. Akad. Wiss. Berlin,' 1861. Pocock.—"Classification of Scorpions," 'Ann. and Mag. Nat. Hist.' (6), xii, 1893. Thorell and Lindström.—"On a Silurian Scorpion," 'Köngl. Svens. Vet. Akad. Handl.,' xxi, No. 9, 1885.

29. Cambridge, O. P.—"A New Family (Tartarides) and Genus of Thelyphonidea," 'Ann. and Mag. Nat. Hist.' (4), x, 1872, p. 413. Cook.—"Hubbardia, a New Genus of Pedipalpi," 'Proc. Entom. Soc. Washington,' vol. iv, 1899. Kraepelin.—'Das Thierreich,' Berlin, 1899. Thorell.—"Tartarides, etc.," 'Ann. Mus. Genova,' vol. xxvii, 1889.

30. McCook.—'American Spiders and their Spinning Work,' 3 vols., Philadelphia, 1889–93.

31. Peckham.—"On Sexual Selection in Spiders," 'Occasional Papers Nat. Hist. Soc. Wisconsin,' vol. i, pp. 1—113, 1889.

32. Moggridge.—'Harvesting Ants and Trap-door Spiders,' 1873.

33. Bertkau.—'Arch. f. Naturgesch.,' vol. xlviii, pp. 316—362; idem, same journal, 1875, p. 235, and 1878, p. 351. Cambridge, O. P.—"Araneidea," 'in 'Biologia Centr. Americana,' vols. i and ii, London, 1899. Keyserling.—'Spinnen Amerikas,' Nürnberg, 1880–92. Pocock.—"Liphistius and the Classification of Spiders," 'Ann. and Mag. Nat. Hist.' (6), x, 1892. Simon.—'Hist. nat. des Araignées,' vols. i and ii, 1892, 1897. Wagner.—"L'industrie des Araneina," 'Mém. Acad. St. Pétersbourg; idem, "La mue des Araignées, "Ann. Sci. Nat.,' vol. vi.

34. Grassi.—"Intorno ad un nuovo Aracnide artrogastro (Kœnenia mirabilis), etc.," 'Boll. Soc. Ent. Ital.,' vol. xviii, 1886.

35. Hansen and Sörensen.—"The Order Palpigradi, Thorell (Kœnenia), and its Relationships with other Arachnida," 'Ent. Tidskr.,' vol. xviii, pp. 233—240, 1898. Kraepelin.—'Das Thierreich,' Berlin, 1901.

36. Bernard.—"Compar. Morphol. of the Galeodidæ," 'Trans. Linn. Soc. Zool.,' vol. vi, 1896, ibique citata. Dufour.—"Galeodes," 'Mém. prés. Acad. Sci. Paris,' vol. xvii, 1862. Kraepelin.—'Das Thierreich,' Berlin, 1901. Pocock.—"Taxonomy of Solifugæ," 'Ann. and Mag. Nat. Hist.,' vol. xx.

37. Balzan.—"Voyage au Venezuela (Pseudoscorpiones)," 'Ann. Soc. Entom. France,' 1891, pp. 497—522.

38. Guerin-Meneville.—'Rev. Zool.,' 1838, p. 11. Karsch.—"Ueber Cryptostemma Guer," 'Berliner Entom. Zeitschrift,' xxxviii, pp. 25—32, 1892. Thorell.—"On an apparently New Arachnid belonging to the Family Cryptostemmidæ, Westw." 'Bihang Svenska Vet. Akad. Handligar,' vol. xvii, No. 9, 1892.

39. Sörensen.—"Opiliones laniatores," 'Nat. Tidskr.' (3), vol. xiv, 1884. Thorell.—"Opilioni," 'Ann. Mus. Genova,' vol. viii, 1876.

40. Berlese.—'Acari, etc., in Italia reperta,' Padova, 1892. Canestrini.—'Acarofauna Italiana,' Padova, 1885. Canestrini and Kramer.—"Demodicidæ and Sarcoptidæ," in 'Das Thierreich,' Berlin, 1899. Michael.—"British Oribatidæ," Ray Soc.; idem, "Oribatidæ," in 'Das Thierreich,' Berlin, 1898; idem, "Progress and Present State of Knowledge of Acari," 'Journ. Roy. Micr. Soc.,' 1894. Nalepa.—"Phytoptidæ," 'Das Thierreich,' Berlin, 1898. Trouessart.—"Classification des Acariens," 'Rev. Sci. Nat. de l'Ouest,' p. 289, 1892. Wagner.—'Embryonal Entwick. von Ixodes,' St. Petersburg, 1893.

41. Bertkau.—"Coxaldrüsen der Arachniden," 'Sitzb. Niederl. Gesellsch.,' 1885.

42. Patten.—"Brain and Sense Organs of Limulus," 'Quart. Journ. Micr. Sci.,' vol. xxxv, 1894; see also his "Origin of Vertebrates from Arachnids," ibid., vol. xxxi.

Authorities not cited by numbers in the text.

Lung Books. Berteaux.—"Le poumon des Arachnides," 'La Cellule,' vol. v, 1891. Jawarowski.—"Die Entwick. d. sogenn. Lunge bei den Arachniden," 'Zeitsch. wiss. Zool.,' vol. lviii, 1894. Macleod.—"Recherches sur la structure et la signification de l'appareil respiratoire des Arachnides," 'Arch. Biologie,' vol. v, 1884. Schneider.—"Mélanges Arachnologiques," in 'Tablettes zoologiques,' vol. ii,

p. 135, 1892. Simmons.—"Development of Lung in Spiders," 'Amer. Journ. Science,' vol. xlviii, 1894.

Coxal Glands. Bertkau.—"Ueber die Coxaldrüsen der Arachniden," 'Sitzb. d. Niederl. Gesellsch.,' 1885. Loman.—"Altes und neues über das Nephridium (die Coxaldrüse) der Arachniden," 'Bijd. tot. de Dierkunde,' vol. xiv, 1887. Macleod.—"Glande coxale chez les Galeodes," 'Bull. Acad. Belg.' (3), vol. viii, 1884. Pelseneer.—"On the Coxal Glands of Mygale," 'Proc. Zool. Soc.,' 1885. Tower—"The External Opening of the Brick-red Glands of Limulus," 'Zool. Anzeiger,' vol. xviii, p. 471, 1895.

Entosternite. Schminkewitsch.—"Bau und Entwick. des Endosternites der Arachniden," 'Zool. Jahrb., Anat. Abtheil.,' vol. viii, 1894. Pocock.—"The Arachnidan Entosternite." 'Quart. Journ. Microsc. Sci.,' vol. 46 (1902), p. 225.

Embryology. Balfour.—"Development of the Araneina," 'Quart. Journ. Micr. Sci.,' vol. xx, 1880. Kingsley.—"The Embryology of Limulus," 'Journ. Morphology,' vols. vii and viii. Kishinouye.—"Development of Araneina," 'Journ. Coll. Sci. Univ. of Japan,' vol. iv, 1890. Locy.—"Development of Agelena," 'Bull. Mus. Harvard,' vol. xii, 1885. Metschnikoff.—"Embryologie d. Scorpion," 'Zeit. wiss. Zool.,' vol. xxi, 1871; idem, "Embryol. Chelifer," ibid. Schimkewitsch.—"Développement des Araignées," 'Archives d. Biologie,' vol. vi, 1887.

Sense Organs. Bertkau.—"Sinnesorgane der Spinnen," 'Arch. f. mikros. Anat.,' vol. xxvii, p. 589, 1886. Graber.—"Unicorneale Tracheaten Auge," 'Arch. f. mikr. Anat.,' vol. xvii, 1879. Grenacher.—'Gehororgane der Arthropoden, Göttingen, 1879. Kishinouye.—"Lateral Eyes of Spiders," 'Zool. Anz.,' vol. xiv, p. 381, 1891. Purcell.—"Phalangiden Augen," 'Zool. Anzeiger,' vol. xv, p. 461.

General works on Arachnida. Blanchard.—"Les Arachnides," in 'L'organisation du règne animal.' Gaubert.—"Recherches sur les Arachnides," 'Ann. Sci. Nat.' (7), vol. xiii, 1892. Koch, C.—'Die Arachniden,' 16 vols., Nürnberg, 1831—1848. Koch, Keyserling, and Sörensen.—'Die Arachniden Australiens,' Nürnberg, 1871–90. Pocock.—'Arachnida of British India,' London, 1900; idem, "On African Arachnida," in 'Proc. Zool. Soc.' and 'Ann. and Mag. Nat. Hist.,' 1897—1900. Simon.—'Les Arachnides de la France,' 7 vols., Paris, 1874–81. Thorell.—"Arachnida from the Oriental Region," 'Ann. Mus. Genova,' 1877–99.

On some New Species of the Genus Phreodrilus.

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With Plates 13—15.

Amongst the Oligochæta obtained by Messrs. Lucas and Hodgson in their recent biological survey of the New Zealand lakes, which they were generous enough to hand over to me for identification,[1] I find two new species of Phreodrilus; and a third species I owe to the kindness of my friend, Mr. Smith, of Ashburton, from whom Mr. Beddard received the type species of the genus.

The characters of the genus, founded by Beddard in 1891, for this New Zealand worm, and hitherto represented there by the single species P. subterraneus, have recently received an extended interpretation by Dr. Michaelsen (5), so as to include the four species of South American worms originally placed by Beddard (3) in a distinct genus, Hesperodrilus; this enlargement of the genus has been rendered necessary by the discovery of a fresh-water worm in Kerguelen which in certain respects bridges over the anatomical gap between the two genera as formulated by Beddard, just as it serves as a stepping-stone in the geographical distribution of the genus as now extended.

It is true that in the type species, as well as in a second species here to be described, there is a peculiarity about the

[1] An account of this collection will be found in P. Z. S., 1903.

male efferent apparatus that does not exist in the South American nor in the Kerguelen species, and one is tempted to regard the existence of this "atrial sac" as diagnostic of the genus as originally formulated, but the intermixture presented by other characters is so involved that it seems better to adopt Michaelsen's view and place them all in the single genus Phreodrilus.

The new species described in the present contribution are (a) P. lacustris, (b) P. beddardi, and (c) P. mauiensis.

Phreodrilus lacustris, n. sp.

A small thin worm, usually much coiled in its preserved state. One individual when stretched measured 20 mm., with a diameter of $\frac{1}{4}$ mm., or even less. This individual consists of seventy-five segments.

The prostomium is prolobic, short, and conical (Pl. 13, fig. 1). Segments I to VII are very short and distinctly biannulate; the following segments are longer, and the annulation less evident. A "lateral line" is very well marked in the stained entire individual.

Chætæ.—The dorsal bristles are solitary, very delicate, and simply capilliform; they are not very long, measuring 0·1 mm., and project for a distance equal to about one quarter to one third the height of body, and are scarcely as long as half a segment. They commence on the third segment, as seems to be the typical arrangement in the genus. Here and there I noted a couple of dorsal chætæ, but the second is quite short. The ventral bristles are in couples, and the two differ in size and in the character of the free end, though the differences may readily escape observation. Both are sigmoid, with a nodal swelling and the free end sharply curved (fig. 2); but whereas in one bristle of each couple (*a*) this end is a simple hook, in the other (*b*) there is a minute tooth on the convex curvature of this hook-like tip; in some cases the tooth is absent and a mere difference in the refringency of this margin suggests a tooth. The chætæ of this

second form (*b*) appear to be rather shorter than the former. In several segments, however, towards the hinder part of the worm, both the chætæ have a tooth. There seem to be no "reserve" chætæ. Of the two forms, the simpler (*a*) is the more ventral of the two, while occasionally in the posterior half of the worm one of the chætæ may be absent. These ventral chætæ measure 0·075 mm. in length; they are smaller than in P. kerguelenensis. In the character of the chætæ this species resembles the South American species ("Hesperodrilus"), rather than the Kerguelen or New Zealand species. The ventral chætæ are absent in Segments XII and XIII; in the latter they are replaced, however, by special copulatory bristles (fig. 3), to which reference is made below.

The clitellum encircles the posterior part of Segment XII and the whole of Segment XIII; its margins in sexually mature individuals are well defined; anteriorly it ceases at about the level of the male genital pores (Pl. 13, fig. 4). In whole specimens, viewed by transmitted light, this region appears blackish, and the epidermis is at least twice the thickness of that layer in the neighbouring regions.

The male pores are on the twelfth segment, close to its hinder margin, in line with the ventral chætæ.

The oviducal pores are immediately behind them on the boundary between the Segments XII and XIII.

The spermathecal pores are in Segment XIII, in front of the line of ventral chætæ.

Thus, the three pores are unusually close together: and the body of the preserved worm is nearly always abruptly bent at the thirteenth segment.

Internal Anatomy.

The alimentary canal presents no noteworthy features; the buccal region is very short; and the pharynx occupies part of Segment II and the whole of Segment III. Its roof is pouched, and the musculature is but feeble. It is remarkable that in

the species recently described by Dr. Michaelsen (5, p. 139), P. kerguelenensis, there is a "schlundkopf" similar to that in Enchytræids; this is certainly not the case in P. lacustris. The œsophagus, immediately behind the pharynx, is dilated, but short, as the Septum III/IV is thrust back by the pharynx nearly to the level of IV/V. The œsophagus passes back into the ninth segment (or tenth, in one case), being constricted at each septum and moderately distended segmentally. Its epithelial cells are, as usual, tall and ciliated.

In the ninth (or tenth) segment the gut suddenly changes its character; the epithelium becomes flatter, and its diameter greatly increases so as nearly to fill the cœlom; it is here filled with dirt, and is constricted, though not deeply, by the septa.

I have but few notes on the vascular system, owing to the imperfect manner in which the vessels can be traced by sections alone. But in entire specimens, viewed by transmitted light, the following facts were recorded:

There are undulating commissural vessels putting the dorsal and ventral trunks into communication in Segments V, VI, VII, VIII, but I could not detect any enlarged hearts nor a blood-gland, either in mounted individuals or in sections.

Excretory System.—In several species of Phreodrilus there appears to be only one pair of pregenital nephridia, but these extend through several segments, as Beddard pointed out (4) in his account of P. (Hesperodrilus) albus, and as Michaelsen finds in P. kerguelenensis; but in P. subterraneus, the only species hitherto described from New Zealand, Beddard (2) apparently did not note any nephridia anteriorly to the fourteenth segment. In the present species I observed in two series of longitudinal sections a nephridium in Segment VII and another in Segment X, but I was unable to trace any connection between these, or to see either funnel or pore. The extreme tenuity of the species renders the tracing of so delicate a tube as a nephridium very difficult.

In P. albus the single nephridium in each side extends from Segment V (in which the funnel lies) to Segment X, its pore, however, being in Segment VI. In P. niger it extends from VII to IX, and in P. kerguelenensis from VII to X. It is probable, therefore, that in this new species —P. lacustris—the pregenital nephridium has the same disposition as in the last-named species.

Reproductive System.—Of the fairly numerous individuals obtained, only three turned out to be sexually mature. One of these was mounted entire, a second was cut into longitudinal sections, and the third was dissected notwithstanding its small size; it was bisected in the region of the reproductive organs, and the latter were partially isolated by removal, under a dissecting lens, of the gut and part of the body-wall, so that the true form and disposition of the spermiducal gland could be studied. In this way a check on the longitudinal sections was obtained, and a very necessary check in the case of an organ, serial sections of which had to be studied under very high powers.

The single pair of testes lies in Segment XI, attached to the anterior wall. Beddard, in his account of P. subterraneus, emphasises the point that in that species the testes extend through or below the septum into the preceding segment. However that may be, it is not the case in P. lacustris, nor, as Michaelsen insists, in P. kerguelenensis. An explanation of the condition described by Beddard seems to be that the septa in this part of the body are very imperfect, as is evidenced by the presence of sperm-morulæ and bunches of developing sperms in several of the segments preceding the eleventh. I find, in one case, that Segments VIII and IX are filled with sperms; in other cases the cœlom of Segments X, XI, and XII is similarly occupied, while in a third individual even Segment VII contains a few of these developing sperms. There are no sperm-sacs; the sperm-masses are free in the cœlom in Segments VII to XII; while I did not find any in the post-clitellar segments It is interesting to recall that also in P. kerguelenensis

the Segments VII to XI are occupied by sperm-masses, and none are recorded posteriorly; whereas in the South American species Beddard finds the "sperm-sacs" extending backwards to Segment XX in P. albus, or even to the twenty-sixth segment in P. niger, and makes no mention of pre-clitellar sacs; but in P. branchiatus he states that no special sperm-sacs exist, but Segments VII to IX are filled with developing sperms. Thus a second possible generic difference is seen to be negatived.

The efferent apparatus (fig. 5) is perhaps the most interesting anatomical feature in the worm of this genus, as Beddard has emphasised in his memoir (2). The funnel of the sperm-duct is a circular, flattened disc, carried on septum XI/XII, but not projecting freely into the segment, as is most commonly the case, for its margin is surrounded by a circle of non-ciliated cells which are continuous with the septum. It is true that the septum here bulges forwards (in sections), carrying the funnel a little distance into the cavity of the segment, but the funnel itself is morphologically flush with the anterior surface of the septum. The cilia covering the funnel are quite short, though in examining the entire individual I at first mistook a bunch of sperms for long cilia, and in sections a similar mistake may readily be made. The sperm-duct passes away from the centre of this flat funnel, and after a few convolutions in a dorso-ventral direction immediately behind the septum, passes backwards towards the hinder wall of Segment XII; it here enters a conical organ, which is a sac (*p. s.*) enclosing a protrusible penis. At the point of entry the sperm-duct is joined by a great spermiducal gland (*gl.*), which is cylindrical and somewhat coiled, or perhaps one should say undulating. The spermiducal gland occupies the whole of Segment XII, and even pushes backwards the posterior septum of this segment. It diminishes in diameter before its junction with the sperm-duct, and this narrow region may be termed the neck (*n*).

The gland has a structure similar to that described by Beddard for P. albus, and to the "appendix of the vas

deferens" in P. subterraneus, which, as Michaelsen has recently pointed out, is nothing else than a spermiducal gland or "prostate" of some authors. The wall of the gland is formed of large rounded cells (seen superficially in fig. 6 and in transverse section at A, fig. 7), with finely granular cytoplasm, outside which is a layer of flat peritoneal nuclei (*c. e.*), but I am unable to detect any muscular coat.

At its proximal extremity, i. e. the neck, the glandular cells gradually cease (fig. 7), and the epithelium becomes quite low and the nuclei flattened; and both cytoplasm and nuclei are much more deeply stained (in borax carmine) than in the rest of the gland. It is into this neck that the sperm-duct enters (fig. 6).

The common duct, or atrium as it may be conveniently termed, now perforates a cylindrical penis, which when at rest lies enclosed in a penial sac (figs. 5, 6). The orifice of the atrium (*op.*) is subterminal.

This penis—which in one specimen was partly protruded through the male pore (figs. 4, 6)—consists of the following tissues:—An internal epithelium, a muscular coat, and an external epithelium. The lumen is small, and the internal epithelium secretes a cuticle, and is not ciliated. The cells are low, with large round nuclei; the muscles (*mp.*) are both circularly and longitudinally arranged (perhaps in reality they are oblique), and outside is the more cubical epithelium, with larger oval nuclei. This epithelium is continuous at the base of the penis with the lining of the penial sac (fig. 7). These latter cells are, when viewed superficially (fig. 6, *e.*), more or less hexagonal in form, and arranged in circular rows with some regularity. The lining of this sac extends up to the epidermis, and round the pore there is an abrupt change in the character of the cells (fig. 7). Outside them is a layer of circular muscles, but I cannot detect any peritoneal cells covering them. The longitudinal muscles of the body-wall are connected with the base and side of the apparatus, and they appear to pass round the penial sac to form part at least, if not the whole, of its muscular coat, which dies out before the

external pore is reached. Protrusion of the penis appears to be effected by the contraction of these muscles.

In comparing this apparatus with that of other species it will be recognised that it closely agrees with that of P. niger, in which the gland occupies the whole segment and pushes back the hind septum. It is much larger than that of P. albus, which is only half a segment long; while the organ in P. kerguelenensis differs from all these in that the sperm-duct apparently enters the spermiducal gland some little distance from the free end; further, this species is without a penis; at any rate, Michaelsen neither describes nor figures any such a termination to the atrium. But the South American species are provided with a protrusible organ, and agree in this matter precisely with P. lacustris.

The ovary lies in Segment XII, alongside of and partially embraced by the coils of the spermiducal gland; the oviduct has the normal structure. In one specimen I noted ova in Segments XV and XVI, but did not detect any ovisacs.

The spermatheca (figs. 8, 9, 10) is an elongated organ, and, as in P. albus and others, is differentiated into three regions, though they are not so sharply marked as in that species. The external pore in Segment XIII, on the ventral surface, leads into a narrow duct (*a*) with a muscular coat; it is only slightly dilated at the entrance—in contrast with P. kerguelenensis and others,—and soon narrows; the muscular coat is longitudinal, and I did not detect any circular fibres in the first part of the duct, which takes a vertical direction obliquely backwards towards the hinder septum; it then bends abruptly upon itself, and here circular muscles appear and the longitudinal muscle-fibres pass onwards beyond the bend, to be inserted in the body-wall (see fig. 10, *lm.*). This second region of the muscular duct (*a'.*), after a short course parallel to its former course, bends backwards and downwards towards the lower part of the septum (XIII, XIV), and passes through the septum and becomes the second region (*b.*)—the muscular coat is here absent, the epithelium becomes lower and appears to be glandular, as the

cytoplasm is very granular and takes the stain deeply; in the entire isolated specimen the cells are vesicular in form, but in sections (fig. 9, *b*.) they appear quite low. This glandular region passes directly backwards below the gut, and gradually opens out to form the ampulla (*c*.), the epithelium of which is flat; this ampulla occupies Segments XIV and XV, being slightly constricted by the septum, and is filled with ripe spermatozoa; there is no spermatophore.

The absence of a distinctly dilated sac at the external end of the duct, opening to the exterior, is in contrast to the arrangement in other species. Lying close to and behind the aperture of this spermatheca is a peculiarly modified chætal sac, containing two copulatory chætæ (figs. 3, 9). This organ is ovoid, or subglobular; its wall is formed of long cells, containing very fine granules; these cells are arranged with their longer axes directed obliquely to the lumen, with the nuclei at their bases.

The "copulatory chætæ" are more delicate than the normal ventral chætæ, and the free end is sharply curved and more hook-like.

In the entire individual the area of skin surrounding the spermathecal pore and the copulatory chætæ is depressed, so that the two organs appear to open together (fig. 3), but longitudinal sections show their true relation as above described (fig. 9). Only in P. kerguelenensis has such an apparatus been hitherto described; and Michaelsen's account differs in two points from the above; firstly, he finds only one chæta in each organ; secondly, the latter opens in common with the spermatheca. The form of the bristle in his fig. 1 is similar to that of P. lacustris. It is noteworthy that Beddard expressly states that there are no copulatory chætæ in the South American species; otherwise one might have been tempted to lay stress on this feature as of generic importance and associated with the absence of a muscular atrial sac.

Localities.—This species was obtained from Lakes Wakatipu (Hauls 20, 23, 25) and Manapouri (Haul 1),

both in the South Island of New Zealand. The hauls were made in depths from 150 to 1000 feet, but I have no information as to whether the worms came from the bottom, though this is probably the case.

Phreodrilus mauiensis, n. sp.

Obtained from Lake Taupo, in the North Island. One individual, unfortunately immature, belongs to this genus, but its characters are just sufficient to define the species. It is evidently distinct from P. lacustris and from P. subterraneus.

The length is 18 mm., its breadth $\frac{1}{2}$ mm.; it contains seventy segments, and is thus twice as stout as P. lacustris, but not nearly as large as P. subterraneus.

The prostomium is large and rounded.

The dorsal chætæ, commencing in Segment III, are capilliform and usually solitary, though frequently a shorter one accompanies the larger.

The ventral chætæ are in couples, of two kinds (Pl. 14, fig. 11), *a* is simply hooked terminally, while *b* has a distinct but small tooth on its upper convex curvature. This chæta is also much larger than the former. The tooth is much more evident than in P. lacustris, and the chætæ are altogether of larger size, being 0·135 mm. in length.

Of internal organs, the testes are present in Segment XI, and small ovaries in the twelfth, in which segment there is also the early rudiment of a sperm-duct; at least, so I interpret a longitudinal cord of cells lying in this segment.

The vascular system presents a feature not observed in P. lacustris, viz. a large "heart" in Segment X, and a somewhat convoluted, or at least lobulated, organ in XI, which appears to be of the same nature as the "blood-gland" described by Beddard in Segments XII, XIII of P. subterraneus. This specimen was stained and mounted, and I have not studied it by means of sections.

Phreodrilus beddardi, n. sp.

While working on the previous species I was naturally led to institute comparisons with the male efferent apparatus in P. subterraneus. I was puzzled to account for the great differences that exist between the complicated arrangement as described and figured by Beddard and the much simpler condition of the organ in P. lacustris.

In P. subterraneus Beddard (**1, 2**) describes the apparatus essentially as follows[1]:—The vas deferens, after a few simple convolutions, unites with a "blind diverticulum" of glandular structure, and the common duct thus formed is very much convoluted—at first a narrow tube agreeing in structure with the vas deferens, it later becomes much wider and different in structure; this tube opens externally without any penis. But this highly convoluted common duct is enclosed in a peculiar muscular sac—the "atrial sac,"—the wall of which is over a considerable distance separated from the wall of the contained tube, but towards the external pore becomes adherent to the latter. In the closed space thus formed Beddard finds loose "ripe spermatozoa," together with "free nuclei," which have, according to him, no relation to the "sperms."

Both the vas deferens and the "blind diverticulum" perforate the wall of the atrial sac near its upper end. The lower extremity of the atrial sac, where its wall adheres to the duct within, is bent upon the previous region, and perforates the body-wall without giving rise to any penial structure.

The arrangement in the species (P. lacustris) that I was studying at the time agrees closely with the male apparatus of certain South American and Falkland Island worms described by Beddard under the generic title Hesperodrilus; and with some of the species, P. albus and P. niger, my species presents several other points of agreement (as noted in the above account). While thus engaged I received Dr.

[1] I do not quote his own words.

Michaelsen's recent memoir, above referred to, on Phreodrilus kerguelenensis, in which he brings forward evidence in favour of uniting the genus Hesperodrilus with Phreodrilus; but, curiously enough, the type species, P. subterraneus, presented apparently so totally different a male apparatus that it seemed to be of great importance to re-examine this form.

I had, as I thought, a specimen of P. subterraneus, collected by Mr. W. W. Smith, who had furnished Beddard with his material. My specimen was labelled "P. subterraneus" by Mr. Smith, who is a keen observer of Oligochætes. I therefore proceeded to examine the male apparatus, but the result of my investigation differs, in certain points, so greatly from Beddard's account of P. subterraneus that I am compelled to employ a new name for the worm. As I was for the moment interested only in the male apparatus, on the assumption that the worm was identical with Beddard's species, I made but a cursory examination of the individual, in order to add any facts that the mature specimen might present, supplementary to those recorded by Beddard for his immature individuals.

I propose for this new species the specific name, P. beddardi, in reference, I need hardly say, to my friend, who has done so much to elucidate the Oligochætal fauna of New Zealand.

My specimen measures 40 mm. by 1·25 mm. It is thus rather shorter than P. subterraneus, specimens of which, he says, "measure about 2 inches."

I counted seventy-eight segments.

The dorsal chætæ visible, as Beddard noted, to the naked eye are, like the ventral chætæ, carried on slight prominences; and the muscles of each bundle are relatively of great size, as they spread out so as to extend almost the entire length of a segment (Pl. 14, fig. 14). The dorsal chætæ commence in Segment III; they are absent from Segment II, as in most other species of this genus. Beddard makes no reference to this absence in P. subterraneus, though in a

later paper describing species of Hesperodrilus this absence is noted. It seems likely that a re-examination of the species will show that it agrees with the rest on this point.

As a rule a single chæta alone projects, but its base is supported by a couple of minute bristles within the follicle; and here and there I note that one of these smaller chætæ are elongated, and though not attaining the length of the normal one, yet project some distance alongside of it.

The ventral chætæ agree precisely with the account given by Mr. Beddard; there is no trace of a "tooth" on the convex side, such as exists in most of the other species of this genus, and in this respect the worm agrees with P. kerguelenensis. In the anterior segments the chætæ are pointed, but posteriorly this point is in many cases worn down, so that the bristle terminates bluntly. The length of the ventral chætæ is 0·3 mm.

The clitellum (fig. 12) is fully developed, and is confined to Segment XIII, with a sharply marked margin anteriorly and posteriorly, but when viewed under a lens the body-wall of Segments XI and XII also appear opaque; when bisected, however, and the cut wall examined, the epidermis in Segment XIII is seen to be very much thicker than that of the neighbouring segments.

The male pore, situated close to the hinder margin of Segment XII, is carried on a slightly everted papilla, in line with the ventral chætæ, which are absent in this segment (fig. 13). In the foot-note on page 290 of his memoir Beddard records that in a mature specimen "one of the segments in the neighbourhood of the thirteenth was furnished with a pair of tubular processes." This I take to be the penial sac referred to below.

The spermathecal pore is, under the lens, a very noticeable vertical slit with distinct cuticulated margin, situated near the anterior edge of Segment XIII, in line with the dorsal chætæ (here absent), as Beddard stated to be the case in P. subterraneus. Though the dorsal chætæ are absent, the chætigerous sac persists (fig. 12, *d'*). The figure, 13 of

Beddard's memoir, illustrating the external anatomy accompanying the description of P. subterraneus is misleading, for, although he rightly states in his text that the pore is in Segment XIII, it is unfortunately figured in Segment XIV.

In external features, then, this new species agrees with Beddard's account of his species.

So far as the internal anatomy is concerned, my further notes deal only with the reproductive organs, to which my attention was more specially directed.

There is no sperm-sac, but the Segments VIII, IX, X, XI are filled with developing spermatozoa.

The spermatheca (fig. 14A) extends through Segments XIII to XVIII, as in P. kerguelenensis and P. albus.

The slit-like aperture leads into the broader end of a large pyriform sac with very thick muscular wall; near the hinder end of the segment the neck of the sac passes gradually into a narrow duct, also with muscular wall and tall epithelial lining; this duct (*a.*) passes backwards through Segments XIV, XV, XVI, lying either above or below the gut, undulating slightly, and then opens into a much dilated, thin-walled sac (*c.*), the "ampulla" of Michaelsen, which lies in Segments XVII, XVIII, the septum between which nips the ampulla which is filled with ripe sperms. There is no sign of any spermatophore, the absence of which seems to characterise the genus.

We do not know the fully developed spermatheca of P. subterraneus, but that of the present species differs from that of P. lacustris in the absence of the glandular region of the duct and in the extraordinary thickness of the muscular coat near the pore (fig. 15), in which both longitudinal and circular fibres take a share.

The Male Efferent Apparatus.—For the purpose of studying this, I had bisected the worm in the neighbourhood of the reproductive organs; from one side of the body I dissected away the male apparatus; the other side, with apparatus uninjured, was cut into a series of transverse

sections. In the former I first studied the apparatus while still in situ in its half of the body as an opaque object; it was then gently removed from its attachment to the body-wall near the pore, and later cleared in glycerine, in which it was possible to turn it over and examine first one side, then the other. Finally, it was stained and mounted in balsam. But, as is known to students of the Oligochæta, the glycerine preparation is of greater value in tracing out ducts, etc., than the balsam preparation.

The entire apparatus is shown in fig. 16, which represents a combination of views of the two sides, obtained by the above methods of study. It is strikingly different from, and altogether simpler than that of. P. subterraneus.

The flat circular funnel rests against Septum XI/XII; the sperm-duct, after passing through the septum, winds to and fro in rather a complicated course in between two of the limbs of a blind glandular diverticulum, or spermiducal gland, which is curved in the form of an S, and its free end lies close behind the Septum XI/XII.

The sperm-duct joins the opposite extremity of the gland, also near the anterior wall of the Segment XII.

Thus far there is a fairly close agreement with Beddard's figure and description; but it is in regard to the contents of the muscular sac that the present species differs from his species.

This "atrial sac"—as Beddard terms it—is bound to the ventral body-wall by numerous muscle-fibres (*mw.*), which radiate from the body-wall and encircle the sac; it contains a tube, or "atrium," resulting from the union of the gland and the duct; but this atrium differs from that in Beddard's species in the following points:—(a) It is of practically uniform diameter throughout its course; (b) it is relatively short, (c) and is only slightly convoluted; while, finally (d), it terminates in a distinct penis, which projects into a "penial sac" or sheath, which in turn communicates with the exterior at the male pore.

There is in the present species no trace of the much convoluted, narrow continuation of the sperm-duct within the atrial

sac, such as is seen in Beddard's fig. 7, in which the wider and shaded portion there shown seems to correspond with the "uniform tube," or atrium, just described. The atrium, however, in the present species is not absolutely uniform either in diameter or in structure, for at the point where it receives the sperm-duct and the gland it is for a very short distance somewhat narrower than it becomes lower down, and also varies somewhat in diameter along its course. Moreover, the upper coiled portion has the same structure as the sperm-duct; the lower differs in structure. The absence of a distinct penis in P. subterraneus may possibly be accounted for by the fact that the individuals which formed the material for Beddard's memoir were immature; but, as I shall point out below, there appears to be a small indication of this organ.

The histological structure of the male efferent apparatus of the present species agrees in general with the account given by Beddard.

The spermiducal funnel is a flat, circular disc, perforated centrally for the exit to the vas deferens. The cells forming this disc are cubical, and bear quite short cilia; this fact, again, is clear enough in the dissected and isolated apparatus, but in sections it is not quite easy to distinguish between the cilia and the spermatozoa accumulated around the funnel. The spermiducal gland, i. e. Beddard's diverticulum or "appendix of the sperm-duct," consists (fig. 19, *gl.*) of an epithelium surrounding a fairly large lumen, and covered externally by peritoneum. Between the two layers of cells is a thin coat of circularly disposed muscle-fibres, which are readily seen in the glycerine preparation, but are more difficult to recognise in sections owing to the thinness of the layer. The epithelial cells are tall, granular, with a vacuolated cytoplasm,—the minute granules being arranged in a network,—and possess large circular nuclei near their bases.

The spermiducal gland enters the atrial sac at its apex (figs. 16, 18). Close to this point the circular muscular coat becomes thicker. The gland then diminishes in diameter, (fig. 19, *n.*), the cells become more distinctly columnar,

longer, and narrower, and the lumen is considerably reduced in size, while the cytoplasm loses its vacuolar character as the granules became arranged more compactly.

A comparison of my fig. 19 agrees closely with the figure 12 of Beddard's memoir.

The spermiducal gland, after entering the muscular sac, becomes much narrowed to form the neck; this decrease in size continues, and the change in the character of the epithelium is more marked three sections lower down (fig. 21), at the level of the entrance of the vas deferens. The epithelium (at *n*) has become quite low, and the distinctness of the cell outlines has disappeared. The cytoplasm becomes deeply stained, and the nuclei, hitherto circular in outline, both in the sperm-duct (*s. d.*) and in the spermiducal gland (*gl.*), and only moderately deeply stained, now become oval or elliptical, and are quite darkly stained in borax carmine; they are also much more closely arranged than before. This narrowed neck of the spermiducal gland (cf. P. lacustris, and Beddard's figure of P. albus) is of very short extent, occurring only in six or seven consecutive transverse sections of the apparatus.

It is into this short narrow neck of the gland that the sperm-duct opens. The structure of this sperm-duct calls for no particular description, as it agrees with the usual account, except that in comparison with the larger Oligochætes the number of nuclei seen in a transverse section is very small, usually only three or four (fig. 20), they take the stain only very feebly; the cytoplasm is faintly granular; and the cells are of course ciliated.

Passing now to the "atrial sac" and its contained tube—the common duct of gland and vas deferens,—which is conveniently termed the "atrium."[1]. A transverse section of

[1] In his account of P. subterraneus Beddard uses the term "atrium" to indicate only the wide, non-ciliated portion of the tube within the muscular sac—the portion shaded in his fig. 7,—while the narrow, much convoluted (white) canal, which is ciliated internally, into which the "diverticulum" (spermiducal gland) opens, he speaks of as a continuation of the vas deferens.

this region over a great part of its extent exhibits the following features (see fig. 22, which is the sixth section below that drawn in fig. 21):

The wall of the sac consists of a thick coat of circular muscles, covered externally by a flattened cœlomic epithelium, which forms a distinctly recognisable membrane with flattened nuclei.

A considerable space is enclosed by this sac, in which lie the sections of the atrium—one, two, or three, according to the region involved. Passing across this space are numerous muscle-fibres (*r. m.*), the direction of which is for the most part radial. These fibres appear to be developed as processes, or at any rate as fibrous refringent modifications of the cytoplasm of certain cells (*m. c.*) which are attached to the inner surface of the wall of the sac. The nucleus of such a cell is oval, and takes the stain well; the body is only very faintly stained.

Some of these muscle-cells are seen in figs. 22, 23, especially well at *m. c.* The general form of the cell is usually spindle shaped, one extremity of which lies against the wall of the sac while the other is "frayed out" into fibres, which pass across the space to be inserted into the wall of the contained atrium; others pass from one part of the wall to another; others, again, appear to pass from one coil (or section) of the atrium to another.

In some sections, less favourable than this, where portions

The term "atrium" is usually, and it seems to me most conveniently, employed to indicate the tube resulting from the union of the spermiducal gland ("prostate") and the sperm-duct, as I attempted to point out in 1890. This is the sense in which Beddard himself uses the term in his Monograph in reference to Tubifex and others. The terminology of this region, in spite of Beddard's own articles on it, still requires a revision. Neither Beddard nor Michaelsen, in their monographs, appear to be quite consistent in the terms employed. The fact that the upper part of the atrium in Phreodrilus beddardi and P. subterraneus is ciliated is quite in agreement with the condition in Tubifex, of which Beddard writes in his monograph, p. 105, "the elongated atrium has its proximal part ciliated; its distal part not ciliated; the latter forms a protrusible penis."

of the fibres are cut across without either terminal being involved, the appearance may suggest bundles of spermatozoa, especially as the cut ends—facing the microscope—have a greater refringency than the more horizontal portions, and suggest "heads" (fig. 23 *rm'*.).

It is possible that these delicate fibres, radiating in groups in every direction across the cavity of the sac, may have been mistaken for sperms; yet I hesitate to assume that an error of this kind would be made by so accurate and experienced an observer as my friend Mr. Beddard. I merely suggest this explanation of the mystery, for he himself admits it is a mystery that surrounds their presence in this completely closed sac; the more so as it appears to me that the relative size of "head" to "tail" indicated in his figures is not that usual to the sperms of Oligochæta.

The atrium itself exhibits two regions, distinguishable by the character of the epithelium, though not otherwise. The upper region (fig. 22 *at.*) closely resembles the sperm-duct, and corresponds with the narrow white tube in Beddard's species; the lower region is cuticulated, and otherwise contrasts with the sperm-duct, and resembles the shaded portion ("atrium") of Beddard's figure 7.

The epithelium of the upper region is finely granular; no cell outlines are visible, and the round nuclei—more deeply staining than in the case of the vas deferens—are few and regularly spaced. The lumen is fairly large, and shows most distinctly cilia. It might be suggested that these are in reality spermatozoa passing down the canal; but against this interpretation are the facts, firstly, that many of them are arranged vertically to the surface of the cells, from which they can be seen arising, and secondly, the failure to discover any heads, which would of course appear as fairly deeply stained points; but nothing of the sort occurs.

There appears to be no circular muscle-fibres round the atrium; for in such sections as cut it longitudinally or nearly so I cannot detect any cut ends of fibres, but outside the epithelium is a layer of muscle-fibres, with which the radiat-

ing fibres previously described are continuous; these fibres take an obliquely longitudinal course.

The atrium has the above structure for only a moiety, though the greater moiety, of its course; further downwards, towards the exterior, its epithelium gradually changes in character (fig. 23). The cells are lower, the nuclei oval and more closely placed; there are no cell boundaries recognisable, but the extent of the cell is indicated by the undulations of the cuticle. The cilia are now absent, and the cytoplasm secretes a distinct cuticle, which is vertically striated—a fact which is more evident when this cuticle is cut rather obliquely, as in the lower half of the figure.

Further, as the duct enlarges the lumen is consequently wider than before. This widened portion terminates some little distance before the external aperture is reached, and forms a short though evident projection into the terminal region of the atrial sac; this projection—or "penis," as it may be termed—is more readily seen in the preparation of the entire apparatus in glycerine than in transverse sections; but, being aware of its presence, one may distinguish it even in transverse sections.

The structure of this penis is best understood by the study of the isolated apparatus (fig. 17). The atrium, now a very narrow tube, perforates a short truncated cone, whose end projects into a more capacious chamber, which may be termed the "penial sac"; at the end of the cone is the aperture of the atrium itself (*o. p.*). The wall of the atrial sac is seen to be continuous with the base of the penial sac.

This figure may be compared with Beddard's figure 30, which represents a longitudinal section at a point "some distance from the external orifice of the atrium," where "the muscular and peritoneal coats become widely separated from the epithelial layer. At this point the lumen of the atrium becomes suddenly contracted." I think that these sudden changes in character of lumen and relation of the coats of the organ in P. subterraneus represent a small—possibly vestigial, or equally possibly a nascent—penis.

Turning now to the sections through this region. The figures 24, 25, 26 are nearly consecutive, and pass through the atrium where it traverses the penis.

The first figure is nearly longitudinal, as it cuts the atrium at a curve; on the right side of the figure, at *y*, the atrial epithelium appears to be invaginated into the cavity of the tube. As a matter of fact, the lining of the tube is here thrown into longitudinal folds, so that the lumen is more or less reduced. The next section (fig. 25) is the fourth from the preceding, and cuts the atrium nearly transversely. The atrial sac is much reduced in size, and the atrial wall itself is folded (at *y*). The following section (fig. 26) involves the very tip of the short penis, cutting it through rather obliquely. The penis in this individual is less prominent than in the individual dissected (compare fig. 26 with fig. 17), for in it the penial sac was protruded and the penis partially so. The section figured at fig. 26 is taken just where the atrial epithelium is being reflected so as to approach (and on the right side has reached) the inner wall of the atrial sac. In the centre of the section the aperture of the penis is shown.

The epithelium of the atrium is continuous at the pore with that covering the cervical penis, and thus with the lining of the penial sac, and this epithelium retains practically its previous character, but the cytoplasm appears to be vertically striated, especially at the outer (basal) surface, where minute vacuoles as of some secretion can be seen, while the cuticle is thicker than before (fig. 27).

There is here no circular coat of muscle, but to the wall is attached a number of retractor muscle-fibres (as in figs. 17, 28), and outside is a layer of flat peritoneal nuclei.

The epithelium of this penial sac passes up to the lip of the external pore, and is here continuous of course with the epidermis; but there is a sudden change (fig. 28), no transition being apparent, the epidermal cells having flattened oval nuclei, which are much smaller and take the stain very

much more powerfully than the nuclei of the internal epithelium.[1]

A comparison of the apparatus in P. beddardi with that described by Beddard for P. subterraneus shows a close general agreement, both macroscopically and microscopically, and, apart from a possible error in interpreting the radiating muscles within the atrial sac, the most striking difference is the absence in this new species of the long, much convoluted, and very narrow portion of the atrium and the presence of a more pronounced penis. In both of these points P. beddardi forms an interesting intermediate stage between the simple conditions of P. albus and the more elaborate arrangement of P. subterraneus, with P. kerguelenensis as in some respects a link with the former.

The most characteristic thing about two of our New Zealand species is the presence of a muscular sac enclosing the atrium (*i. e.* the common duct of vas deferens and spermiducal gland), which is more or less coiled so as to be stowed away within it. No trace of this sac exists in P. kerguelenensis, but the spermiducal gland is shorter and the "atrium" is much longer than in the South American species, and is moreover glandular, as is the atrium of our New Zealand forms. The absence of a penis in the Kerguelen species forbids us placing it as a direct link. But if we start with P. albus, we find the vas deferens opening into the short, narrow, and apparently non-glandular neck of a small spermiducal gland. (The gland is much larger and coiled in P. niger and in P. lacustris, where it occupies the whole length of its segment). The common duct thus formed perforates a protrusible penis, contained within a comparatively capacious penial sac. The next stage, apart from the penis, is P. kerguelenensis, in which the common duct (atrium) is longer and glandular. Then comes an entirely new structure, and we have the stage in which the muscular wall becomes separated from the glandular epithelium, so as

[1] Unfortunately the lithographer has made the nuclei of the epidermic cells next the pore round instead of flat.

to form a muscular "atrial sac." In P. beddardi the atrium is much larger than in the previous species, and is coiled, and terminates in a small penis. And finally, in P. subterraneus the atrium becomes drawn out to an extraordinary length, and is differentiated into a long narrow, and a short glandular region, while the penis is quite small.

Mr. Beddard, in his memoir on P. subterraneus, has called attention to the peculiarity and unique character of this atrial sac, and has compared it with certain other structures, and the inclusion of Hesperodrilus in the genus renders it easier to make comparisons with the closely allied family, the Tubificidæ.

In Tubifex itself there is a comparatively simple protrusible penis, surrounded by a muscular wall, forming a sac in which it lies. This is quite comparable to the arrangement in P. lacustris. But in other genera, such as Limnodrilus, the muscular investment extends much further up the wall of the apparatus; the penis is much more powerfully developed it is true, but we do not know to what extent it can be protruded. From its general structure one is inclined to think that the extent is limited. This muscular sheath, which in some species (constituting the genus "Camptodrilus" of Eisen) is formed of spirally arranged fibres, surrounds a considerable length of the "atrium," narrowed of course as compared with the saccular region higher up the tube. It seems to me that the atrial sac of Phreodrilus beddardi is a step further than this, in which the whole atrium has become surrounded by muscle.

It appears that protrusion in this case is effected by the compression of the fluid within the sac by the contraction of the muscle in the wall, which is aided by the contraction of the fan-shaped muscle (*m. w.*) above described. This muscle is probably homologous with those surrounding the penial sac of P. lacustris, themselves in continuity with the longitudinal muscles of the body-wall, and acting as "protrusors." In P. beddardi the penial sac itself is provided with longitudinal muscles alone, which appear to act as

retractors (*l. m.*), and perhaps the spiral muscles covering the atrium derived from the radiating fibres (*r. m.*) which connect it with the wall of the atrial sac serve to retract the penis.

Dunedin, April 11, 1903.

Literature.

1. Beddard, F. E.—"Abstract of some Investigations into the Structure of the Oligochæta," 'Ann. Mag. Nat. Hist.,' 1891, p. 88; Phreodrilus, p. 92

2. Beddard, F. E.—"Anatomical Description of Two New Genera of Aquatic Oligochæta," 'Trans. Roy. Soc. Edinb.,' xxxvi, p. 273.

3. Beddard, F. E.—"Prel. Notice of South American Tubificids, etc.," 'Ann. Mag. Nat. Hist.,' 1894 (6), xiii, p. 205.

4. Beddard, F. E.—"Naiden, Tubificiden, und Terricolen," 'Hamburg. Maghal. Sammelreise,' 1896.

5. Michaelsen, W.—"Die Oligochæt. d. Deutsch. Tiefsee Exped.," 'Wissensch. Ergebn., etc.,' 1902, p. 133.

EXPLANATION OF PLATES 13—15,

Illustrating Mr. W. Blaxland Benham's paper "On some New Species of the Genus Phreodrilus."

Letters Employed in the Figures.

a. Muscular duct of spermatheca. *at.* Atrium, either its wall or cavity. *ats.* Atrial sac, either its wall or cavity. *b.* Glandular portion of duct of spermatheca. *c.* Ampulla of spermatheca. *c. e.* Cœlomic epithelium, or its nuclei. *c. m.* Circular muscle, whether of body-wall or atrial sac. *cp.* Sac with copulatory chætæ. *cp. ch.* Copulatory chætæ. *d.* Dorsal chætæ. *e.* Epithelium of penial sac, etc. *ep.* Epidermis. *f.* Funnel of sperm-duct. *gl.* Spermiducal gland. *lg.* Longitudinal muscles of body-wall. *lm.* Longitudinal muscles of other organs. *m.* Muscle. *m'.* Cut ends of muscle-fibres. *m. c.* Body of muscle-cell. *mp.* Muscles of penis. *mw.* Fan-shaped muscle passing from atrial sac to body-wall. *n.* Neck of spermiducal gland. *op.* Orifice of penis. *p.* Penis. *p. s.* Penial sac, either its cavity or wall.

r. m. Radiating muscle-fibres traversing the cavity of the atrial sac. *s.* Septum. *s.d.* Sperm-duct. *spth.* Aperture of spermatheca. *t. m.* Retractor muscles of penial sac. *v.* Ventral chætæ. *x.* Point of union of sperm-duct and spermiducal gland. *y.* Folding of atrial epithelium at the penis. *z.* Transverse fold of the epithelium of the penial sac. ♂ Male pore. ♀ Orifice of oviduct.

Figs. 1 to 10 illustrate the anatomy of Phreodrilus lacustris, n. sp.

Fig. 11 refers to P. mauiensis, n. sp.

Figs. 12 to 28 refer to P. beddardi, n. sp.

FIG. 1.—Side view of anterior extremity of P. lacustris. × 40, camera. Note the conical form of prostomium, the absence of dorsal chætæ on Segment II and the annulation of the segments.

FIG. 2.—The two ventral chætæ of a bundle. × 640. (*a*) The simple form; (*b*) the toothed one.

FIG. 3.—The spermathecal pore and copulatory chætæ of Segment XIII, as seen in a transparent specimen.

FIG. 4.—Ventral view of Segments X to XIV, to show the clitellum and genital pores; the former is shaded. On the right side of the figure the penis (*p.*) is represented as being protruded from the male pore (♂). The ventral chætæ are absent in XII, XIII, but in latter are replaced by copulatory chætæ (*cp.*). ♀ oviducal pore. *spth* Spermathecal pore.

FIG. 5.—View of the entire male efferent apparatus, constructed from sketches of the opaque and transparent preparations of the isolated organ. The apex of the spermiducal gland (*gl.*) is slightly shifted from its true position near the septum, so as to exhibit more clearly the course of the sperm-duct (*s. d.*); the union of the two is indicated at *x*. The wall of the penial sac (*p. s.*) is represented as being transparent, allowing the penis (*p.*) to be seen; the sac is still attached to the body-wall at the male aperture.

FIG. 6.—A somewhat diagrammatic representation of the penial sac, etc., founded on the study of the organ mounted in glycerine. In the upper part of the sac the circular coat of muscles (*m.*) is in focus; in the middle of the sac the bases of the epithelial cells (*e.*) are shown; while lower down the wall is in optical section, so that the cavity (*ps.*) is in view, as well as the external epithelium of the penis itself. The terminal region of the penis is seen in optical median section, which brings into view the lumen of the distal part of the atrium, which opens near the tip of the penis at *o. p.*

FIG. 7.—A median longitudinal section along the penis and its sac, drawn as carefully as possible under Leitz, oil immersion, $\frac{1}{12}$. The spermiducal gland (*gl.*) is seen to change its character as it narrows to form the neck (*n.*), into which the sperm-duct opens (see fig. 5). This neck is continued through the penis, and corresponds to the atrium of some other species. The longi-

tudinal muscles (*lg.*) of the body-wall pass upwards so as to form the coat of the penial sac. In the upper part of the figure, at A, the spermiducal gland has been cut transversely in a wider region.

Fig. 8.—The spermatheca in situ. *spth.* Its orifice. *a.* Its muscular duct. *b.* Glandular region of the duct. *c.* Ampulla. *cp.* Organ with copulatory chætæ.

Fig. 9.—The duct of the spermatheca and the copulatory organ, seen in longitudinal sections. The details of structure are only partly filled in. The copulatory organ (*cp.*), with its chætæ, is seen to open by a pore distinct from that of the spermatheca. *a.* The proximal portion of the muscular duct. *a'.* The recurved portion of the same. *h.* The point at which *a.* passes into *a'.*

Fig. 10.—The point *h.* of the preceding figure, as seen in the entire mounted specimen, under an oil immersion, $\frac{1}{12}$. The region *a.* is seen in optical section, *a'.* in surface view. The longitudinal muscles (*lm.*) surrounding *a.* pass away to the body-wall. The circular coat (*cm.*) around *a'.* ceases at *h.*

Fig. 11.—The ventral couple of chætæ of P. mauiensis. × 500.

Fig. 12.—View of the right side of the genital region of P. beddardi. *d.* Dorsal bristle. *d'.* The chætaless dorsal chætal-follicle on Segment XIII. *v.* Ventral chætæ. ♂ Male pore. ♀ Female pore. *spth.* Spermathecal pore.

Fig. 13.—Side view of ventral region of the left side of part of Segments XI, XII, showing the everted penial sac (*p. s.*). *v.* Ventral chætæ of Segment XI.

Fig. 14.—Optical section of the ventral portion of a segment, showing the ventral couple of chætæ, with their greatly developed muscles (*m.*).

Fig. 14A.—The spermatheca in situ, seen from the side. *spth.* Spermathecal pore. *a.* Muscular duct. *c.* Ampulla.

Fig. 15.—A transverse section through the muscular duct of the spermatheca, a short distance from the pore. It is provided with both circular and longitudinal muscles, the former of considerable thickness; in the latter the round nuclei of muscle-cells (?) are seen between the cut fibres and the peritoneum (*c. e.*).

Fig. 16.—The male efferent apparatus; the figure is constructed from sketches of the isolated organ as seen as an opaque object and in a glycerine preparation. The atrial sac is represented as transparent so as to exhibit the atrium within, and the penial sac also allows the contained penis to be seen. A fan-shaped bundle of muscle (*m. w.*), springing from the body-wall, enwraps the atrial sac, giving rise to its circular coat of muscles.

Fig. 17.—The penial sac and penis in optical section, as seen when isolated in a glycerine preparation. The lower end of the atrium (*at.*) is seen in the atrial sac (*ats.*), to the inner surface of which it is connected by radiating muscle-fibres (*r. m.*); it is also accompanied by longitudinal fibres. The penis, in comparison with the preceding species (fig. 5), is seen to be of much

smaller size, and the orifice is terminal (it is represented too large in the figure).

[N.B.—Figs. 18—28, representing transverse sections of the efferent apparatus, are drawn, under Leitz, oil immersion, $\frac{1}{12}$, as carefully as possible; they are not camera drawings, and consequently they are not all quite of the same relative size.]

Fig. 18.—Cuts through the spermiducal gland at its entrance into the atrial sac; note the circular muscle at one end of the figure.

Fig. 19.—The section immediately following the preceding. It cuts the gland twice; one section (*gl.*) is outside the atrial sac, the other (*n.*) is now within the sac. There is a marked difference in the character of the cytoplasm in the two cases and the size of the lumen.

Fig. 20.—Transverse sections of the sperm-duct.

Fig. 21.—Section involving the entrance of the sperm-duct (*s. d.*) into the neck (*n.*) of the spermiducal gland within the atrial sac (*ats.*). The section is the third below that shown at fig. 19. The epithelium of the neck is formed of low cells, deeply staining, and apparently ciliated.

Fig. 22.—A transverse section through the upper part of the atrial sac (the sixth section below fig. 21) at nearly its widest region; it shows the atrium (*at.*) cut through thrice, owing to its coiling. The epithelium closely resembles that of the sperm-duct, and, like it, is ciliated. *m. c.* Muscle-cells attached to the inner surface of the wall of the sac, and produced into radiating muscle-fibres (*r. m.*) passing to the atrium, which they enwrap in a spiral direction, so that the fibres in places are cut transversely (as at *r. m'.*). The wall of the sac is formed of circular muscles (*c. m.*), which appear to be connected with the muscles (*mw.*) from the body-wall.

Fig. 23.—A section through the atrial sac some distance lower down. The diameter of the sac has diminished. The epithelium of the atrium has altered its character; it no longer bears cilia, and is covered internally by a striated cuticle. The radiating muscle-fibres are cut obliquely on the right, presenting the appearance of short filaments and refringent dots. The parent muscle-cells (*m. c.*) form almost a complete lining to the sac.

Fig. 24.—The twenty-eighth section below that figured at fig. 22. It cuts the atrium longitudinally at a bend near its lower extremity, just before it passes into the penial sac (cf. fig. 17). On the right (*y.*) the epithelium is infolded. The circular muscles of the atrial sac are now seen cut across.

Fig. 25.—The fourth section from fig. 24. The epithelium is seen to be much folded (*y.*).

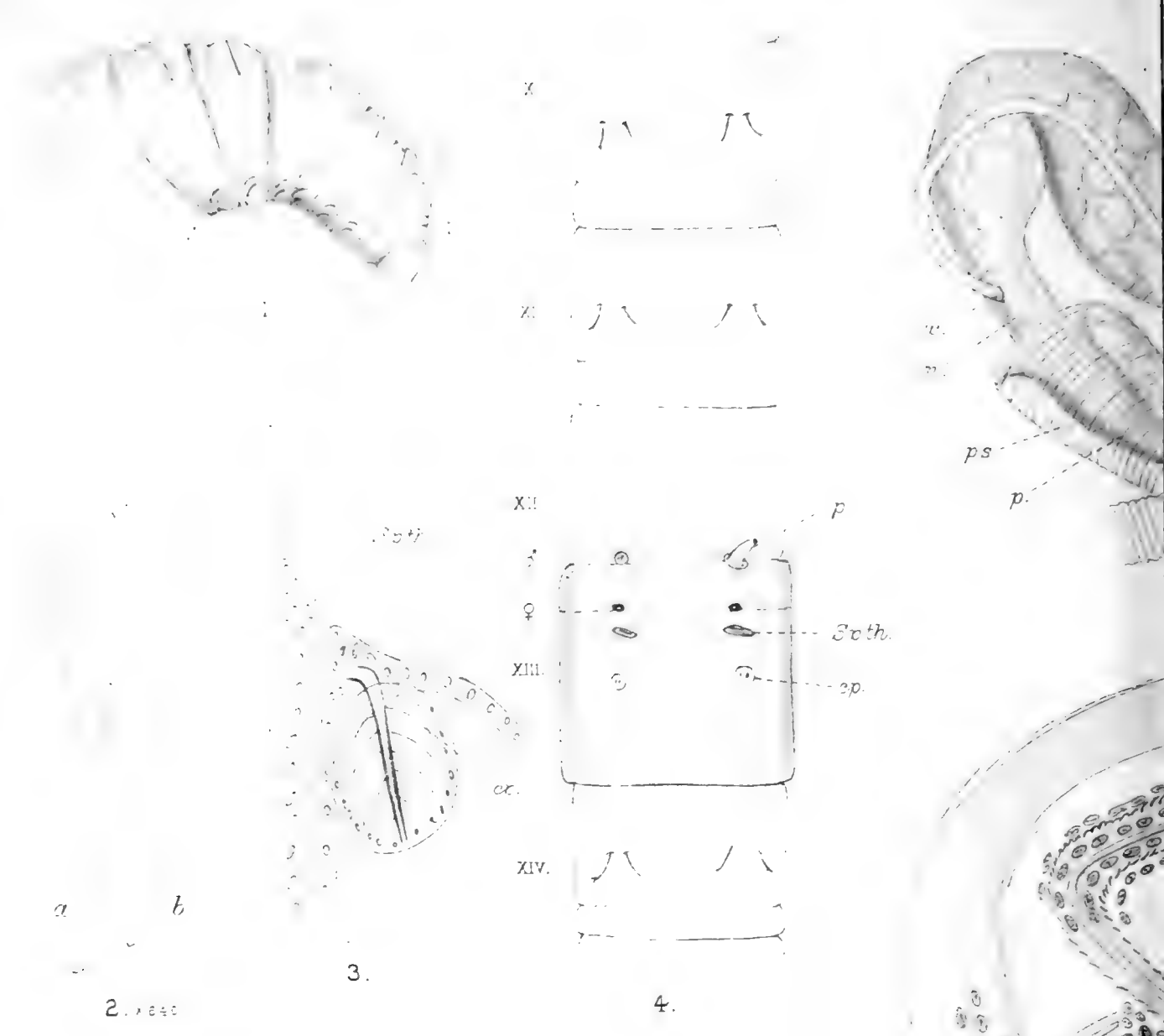
Fig. 26.—The section following that drawn at fig. 25 passes obliquely through the tip of the penis, the aperture of which (*op.*) is seen to occupy the

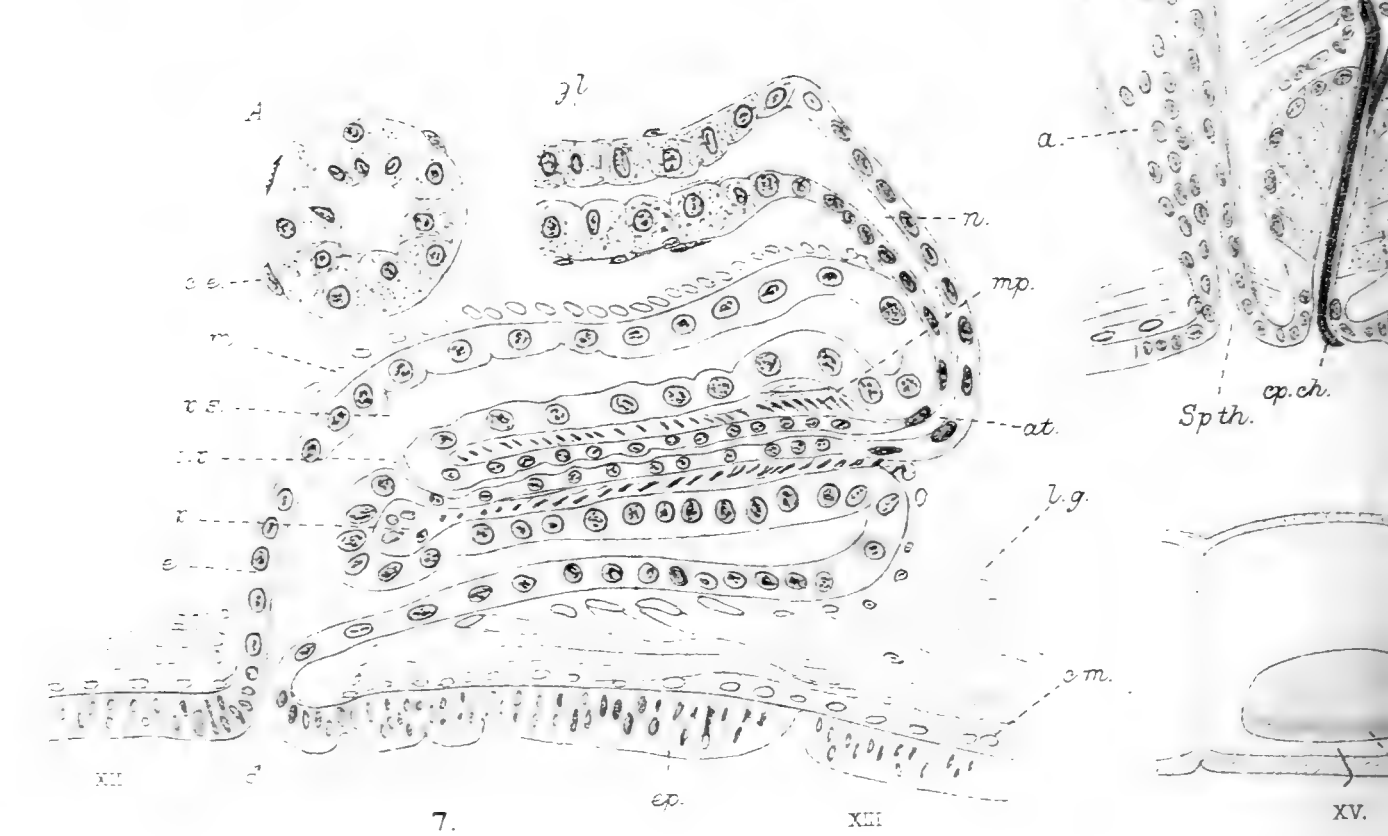
position of *y.* in fig. 25. On this side of the section the lining of the penial sac is involved (*p. s.*); on the left side the cavity of the atrial sac is still seen

[N.B.—The cytoplasmic details are not indicated in figs. 25, 26, 28.]

Fig. 27.—The third section below the last is a transverse section of the penial sac. The epithelium is totally different from that of the atrium; its cytoplasm is striated, and small vesicles are seen in it, especially near its base.

Fig. 28.—A longitudinal section through the penial sac involving the male pore (cf. fig. 17). The fold (*z.*) in the epithelium is evidently connected with the non-protrusion of the penial papilla.





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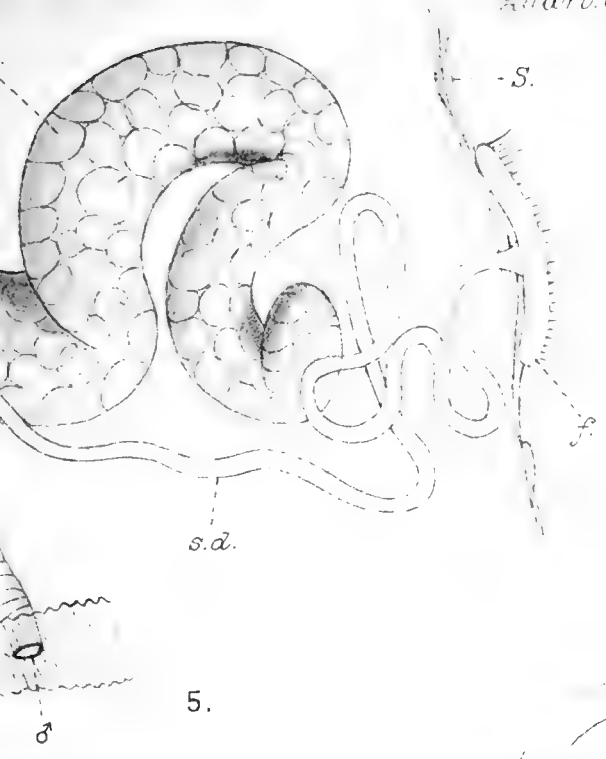

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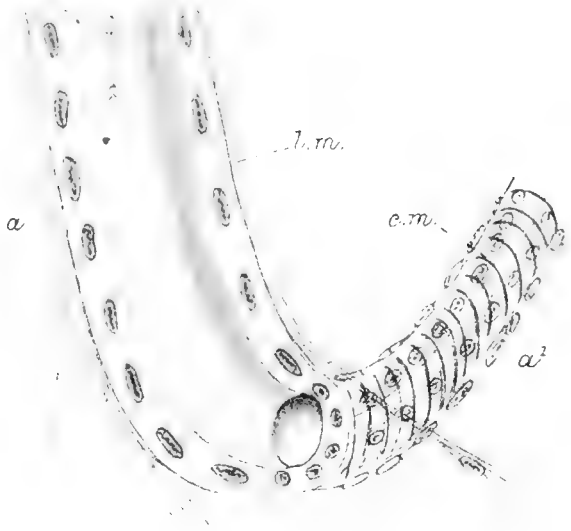

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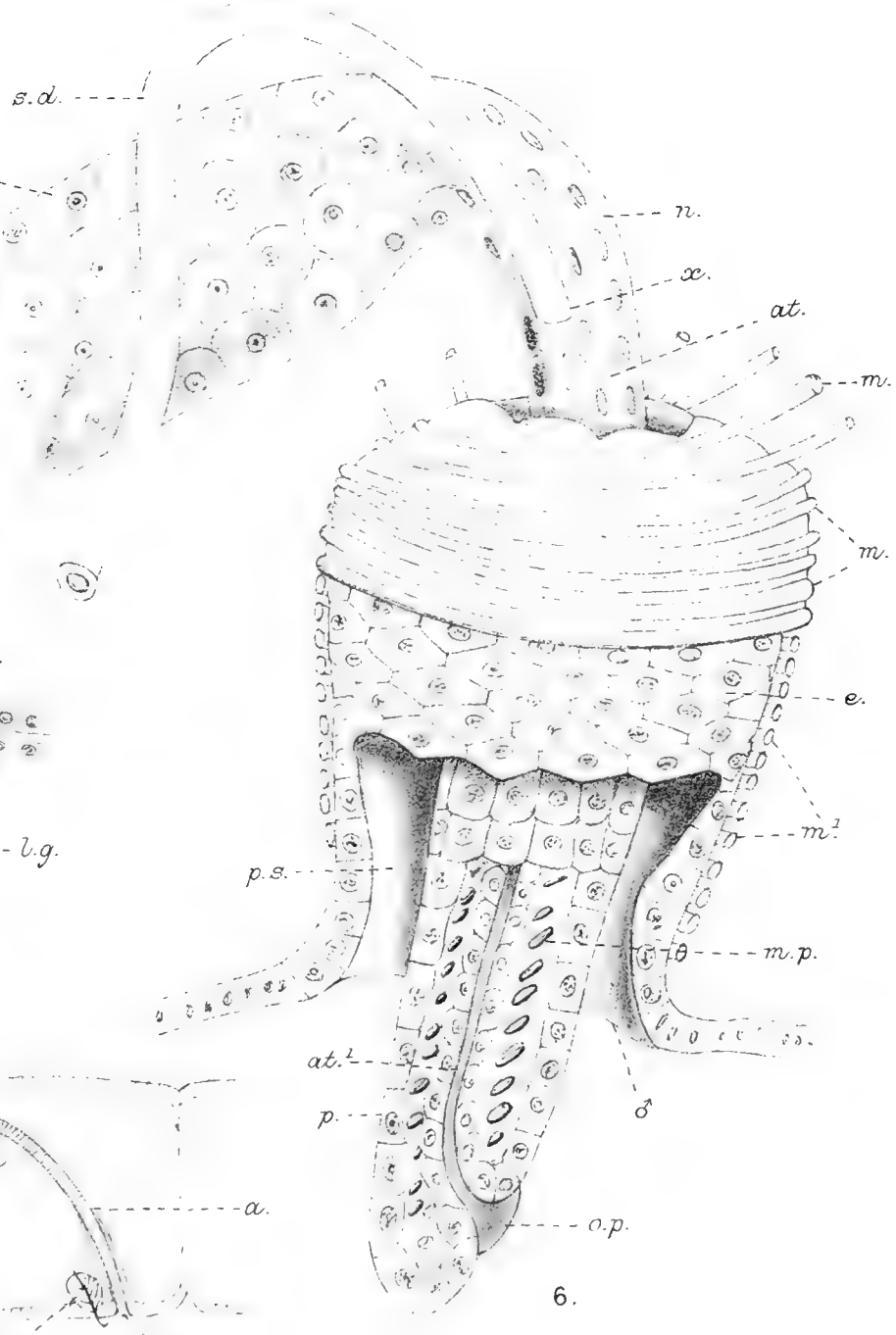

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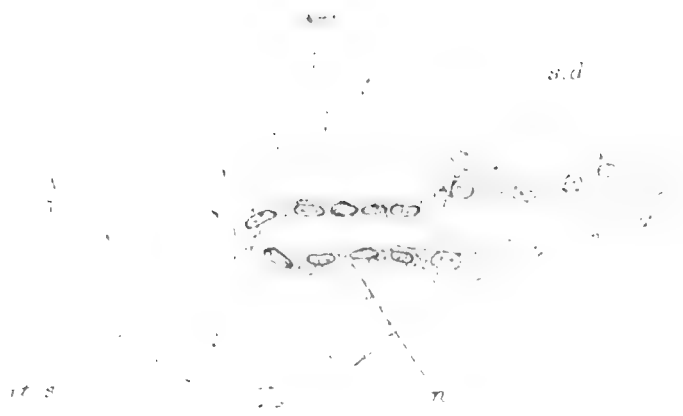

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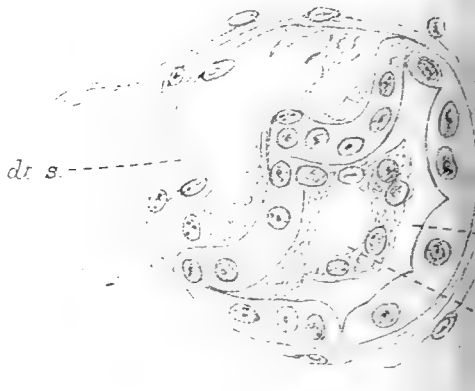

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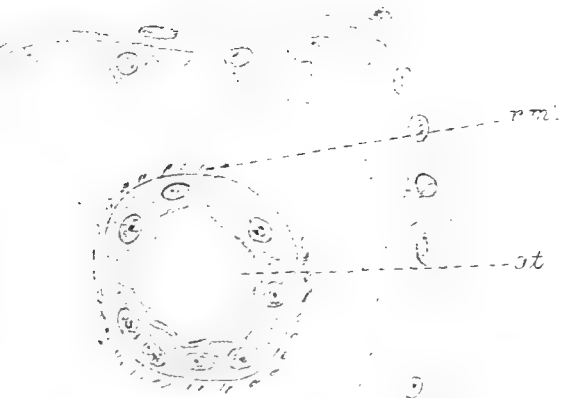

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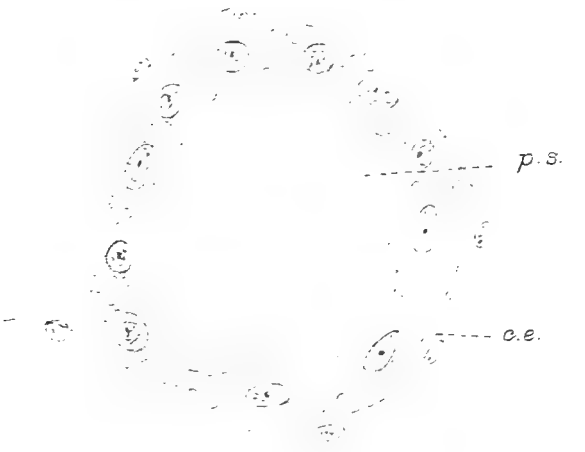

27.

On a New Species of the Genus Haplotaxis; with some Remarks on the Genital Ducts in the Oligochæta.

By

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With Plates 16—18.

Amongst the material collected by Mr. Keith Lucas during his biological survey of the New Zealand lakes I find two small worms belonging to the genus Haplotaxis, of Hoffmeister (= Phreoryctes, auctorum), which differ from the two species already known—H. gordioides, from Europe and America, and H. smithi, from New Zealand—in being provided with only a single pair of ovaries and oviducts. For this new species, therefore, I propose the name Haplotaxis heterogyne. Justification for placing the worms in this genus, hitherto characterised by the possession of two pairs of female organs, will be found below.

The worm is further remarkable and of general morphological interest on account of the very close structural resemblance, I may almost say identity, of the sperm-ducts with the nephridia. This matter also is reserved for discussion till the characters of the new species have been described.

Haplotaxis heterogyne, n. sp.

Of the two individuals one is sexually mature, the other has only the rudiments of the genital organs. The former was

studied at first entire, stained in alum-cochineal, and mounted in Canada balsam; it was then cut into a series of transverse sections. The anterior end of the other was cut longitudinally; a portion from the middle of the body was cut transversely, and other portions of the worm were studied in glycerine.

The prostomium, as usual in this genus, is remarkably long and narrow, but does not exhibit any annulation. The sensory cells form a thick layer over its whole extent. The Segments I and II are short, and the subsequent ones become progressively larger; the body is much dilated in the region occupied by the sexual products (Pl. 16, fig. 1). Each segment is surrounded by a ring of more deeply stained nuclei at about the level of the chætæ, probably a ring of sensory cells; and a lateral line is evident in transverse section.

The chætæ are four in number in each segment (fig. 15). The single dorsal chæta is only about one third to one half the length of the single ventral one, which is very much stouter than the former; both are, however, alike in form —the basal region is straight, the freely projecting portion is curved so as to be sickle shaped with a simple point. In the mid-body the dorsal chæta is about 0·09 mm., the ventral 0·15 mm. in length. The dorsal chætæ are present throughout the worm.

The clitellum covers Segments XI to XIII and part of XIV; it surrounds the body, but is better developed laterally than either ventrally or dorsally, indeed, it appears in transverse section as thinner dorsally than elsewhere.

I was unable to detect any of the genital pores on the entire worm; but from a study of sections I believe that the two pairs of male pores in Segments XI and XII lie just in front of the ventral chætæ. There is a single pair of oviducal pores in Segment XIII; each pore is external to the line of ventral chætæ, and lies below a slightly overhanging projection of the lateral margin of the ventral surface. In the possession of a single pair of female gonads and ducts this species differs from the other two known species, H. gordioides, and H. smithi; hence the specific name hetero-

gyne. The two pairs of spermathecæ open at the anterior margins of Segments VIII and IX.

Internal Anatomy.

Alimentary System.—The buccal region is noticeably long, extending through the three anterior segments of the body; there is no pharynx, but the buccal tube opens into a gizzard in Segment IV (fig. 1, *g*). This organ is very different structurally from a pharynx, for which it may easily be mistaken unless the worm be studied by means of sections.

It is a cylindrical organ, lined by a thick cuticle (fig. 2); the wall is for the greater part of its extent muscular; the muscle is equally developed on all sides, and consists in the main of a thick circular coat, outside which is a layer of longitudinal fibres, together with others intermingled with the outer lamellæ of the circular coat. A distinct cœlomic epithelium surrounds the whole. From its dorsal and lateral walls a few muscle-slips pass to the body-wall.

In the posterior third of the organ the muscular coat diminishes gradually, and the epithelial cells exhibit more or less numerous goblet-cells, the contained secretion of which is not stained by hæmalum; these goblet-cells open by distinct holes through the cuticle.

Such a structure more nearly resembles a gizzard than a "pharynx;" there is no "dorsal muscular pad," such as occurs in Enchytræids, nor is there any "dorsal ciliated pouch," such as is met with in many earthworms as well as most aquatic Oligochætes. The presence of a gizzard in a so-called "limicoline" member of the order breaks down one more of the barriers which were formerly supposed to separate the aquatic from the terrestrial Oligochætes; and it is remarkable that both Haplotaxis gordioides and H. heterogyne, purely aquatic worms, should possess a gizzard, whilst the majority of aquatic species of terrestrial genera lose the gizzard.[1]

[1] In looking up the literature of the subject, after writing out my notes, I

The œsophagus is quite a narrow tube, lined by ciliated epithelium, which is somewhat folded; it passes backwards, below the sperm-sacs, as far as Segment XII, where it is slightly dilated, and the ventral wall thrown into folds, which are more vascular than elsewhere.

As to the vascular system, the dorsal and ventral vessels are connected by a pair of undulating "commissural vessels" in every segment, as in the other two species of the genus.

Nephridia.—The first nephridium occurs in Segment X, with a funnel in the preceding segment; none are present in the following three segments, in which the genital ducts lie, but in Segment XIV and in each of the subsequent segments there is a pair of excretory organs, and these are larger than those in the tenth segment.

In the immature individual likewise no nephridia are to be seen in the Segments XI, XII, XIII.

In H. gordioides Forbes (4) finds rudimentary nephridia in all the genital segments of a quite immature individual in which no trace of genital organs are yet present.

The disposition and structure of the nephridium is illustrated in figs. 3—8. The nephridial funnel of the post-ovarian organ, at least, has the usual form, with one lip a good deal higher than the other (fig. 9); the canal, after piercing the septum, perforates a row of vesicular cells, which form a loose loop. The cytoplasm of these cells exhibits (when studied under a $\frac{1}{12}$ homogeneous immersion lens) a faint network, but immediately around the canal this network is replaced by more closely granulated protoplasm, which forms a distinct but narrow "wall" to the canal (fig. 8).

These cells do not correspond with the vesicular "peritoneal cells" that surround the nephridium in certain earthworms, or which occur, for instance, in Psammoryctes, as figured by Vejdovsky.

find that Michaelsen (5) has already described this gizzard in H. gordioides in much the same terms as I have above used. In this paper he corrects several errors and misconceptions in the description of the various "species" of Haplotaxis, and shows that the European and American species are identical.

I failed to detect any cilia in the lumen of the nephridial canal.

I have not endeavoured to trace out the course of the lumen in detail, but I note that for the greater part of its course its wall is quite simple, *i. e.* is formed by the faintly granular protoplasm of the perforated cells; but at the apex of the loop there is a differentiation of this protoplasm to form a more distinct, apparently striated, boundary to the lumen (fig. 13, *a*), comparable to the wall of the "ampulla" in the nephridium of Lumbricus.

After leaving the funnel the nephridial loop mounts up alongside the gut, and nearly reaches the dorsal body-wall.

The nephridial canal passes to the body-wall a short distance in front of the ventral chæta (figs. 5, 6, 7), passing amongst the chætal muscles to the chætal gap in the longitudinal muscle of the body-wall. Here the structure of the nephridial cells suddenly changes; the cytoplasm is now very highly granular, the cells, or rather syncytium, becoming much more deeply stained than elsewhere; there is no trace of the cytoplasmic network which is observable in the greater part of the nephridium; the nuclei, too, are rather different (figs. 10, 11). This very granular region may, for convenience, be termed the "duct;" but although I traced the nephridial canal thus far, I was unable to detect any perforation of the more superficial granular cells. They pass through the muscular wall into the epidermis, where they spread out slightly; but I could detect no pore.

This "duct" is readily distinguished from the surrounding epidermis by its affinity for the stain, the epidermal cells appear homogeneous, and spaces exist between the bases of many of the cells. The "duct," however, passes right through the epidermis to the surface.

The nephridium in Segment X appears to be in a state of degeneration; it is relatively smaller than the following ones, and the loop only reaches upward as far as the lateral line, though the diameter of the body is here greater than it is more posteriorly (figs. 12 and 13).

The nephridial funnel, lying in Segment IX, is situated immediately in front of the root of the first testis, as shown in the figure of the longitudinal section of this region of the immature individual (fig. 14). The funnel is smaller than that of the post-ovarian nephridium.

I was unable to trace this first nephridium to the body-wall; it was easy enough to follow it upwards to a point close to the body-wall near the lateral line, some little way in front of the chætæ, but there it seems to cease.

It is interesting to find that Forbes was equally unable to find a pore in the case of the first nephridium in "Phreoryctes emissarius."

Reproductive System.—There are two pairs of testes attached to the anterior wall of Segments X, XI respectively, and on the posterior wall of each of these segments is a pair of spermiducal funnels of a simple plate-like form.

The course of the sperm-duct from funnel to the body-wall is shown in figs. 16—24.

Each of the four sperm-ducts leaves its funnel close to the lower or ventral margin (fig. 32), as described by Beddard (1) for H. smithi; it then passes through the septum, and afterwards behind the funnel and outside the following testis; it soon becomes slightly undulating, and reaches to the level of the lateral line; then, bending down, it reaches the body-wall at a point about midway between the margin of the segment and the ventral chæta (figs. 24, 29).

I have been quite unable, however, to detect any external opening in either of the four ducts, and, indeed, only in the case of the left duct of the anterior pair was I able to trace it actually to the body-wall and into continuity with the epidermis (fig. 29).

Owing to the slight obliquity of the sections and to the displacement due to the previous compression in mounting the specimen, the duct of one side is cut transversely, and that of the other side longitudinally in at any rate part of its course (fig. 28), and in this figure both the upward and downward part of the canal are involved. The duct has almost all the appearance of a nephridium, and its general

disposition in the body is similar to that of the more posteriorly placed excretory organs (cf. figs. 3 and 7 with figs. 16—24). Section across it does not show a definite epithelium, but the lumen appears to traverse a single row of cells. These cells, or rather syncytium, for I cannot detect any boundary to the component cells, are not vacuolated as are the nephridial cells, nor is the protoplasm immediately bounding the lumen of the duct specially granular to form so distinct a "wall" as in the case of the nephridium. Indeed, when first examining the sections I mistook the duct for a nephridium, but a more careful examination of consecutive sections, drawn with a camera, shows quite without any doubt that this tube, if it be a nephridium, at any rate acts as a sperm-duct. In the right duct a group of deeply stained spermatozoa can be seen entering the tube (fig. 32), which, as stated above, starts from the ventral edge of the funnel. In the lumen of the left duct I see a bunch of sperms some distance away from the funnel; these appear both in a portion of the duct cut transversely (figs. 25, 26) and a little further along, appear in a longitudinal section at a bend in the duct (fig. 27), and they can be traced through several consecutive sections. These sperms are deeply stained by the hæmalum, and show up perfectly unmistakably.

In this connection it is interesting to recall the fact that the earlier students of Haplotaxis gordioides believed that the nephridia of these segments acted as sperm-ducts, but Mr. Beddard was the first to identify true genital ducts in the genus in his examination of H. smithi; he describes (**1**, p. 391) the duct as "a ciliated tube composed of a single layer of columnar cells," and his figure 6 (pl. xxiii) illustrates this statement.

However this may be in H. smithi, the sperm-duct in the present species can scarcely be distinguished structurally from a nephridium, except that the margin of the canal is a little more distinctly marked in the latter, and the cytoplasm of the cells is vacuolated, and the canal is more convoluted than in the sperm-duct, in which, too, cilia can be seen dis-

tinctly in most of the sections. These points of difference require very high magnification, and are not recognisable without a homogeneous immersion lens. But if there is a close similarity between the excretory and genital ducts, there is an immense difference between the spermiducal funnel, with its high ciliated cells forming a conspicuous, broad, thick disc on the septum (fig. 31 et seq.), and the minute nephridial funnel just projecting through a septum.

In the Segments XI, XII I find no nephridia—no tubes, i.e. besides the sperm-ducts,—nor is there any funnel belonging to these tubes other than the flat, wide sperm-funnels. Even in the immature worms no nephridial funnels exist alongside the young sperm-funnels (fig. 37).

It is a curious fact that the sperm-ducts, even in a worm in which ripe sperms fill the sperm-sacs as well as the spermathecæ, and with large ova in their proper segments, should be so difficult to trace; Michaelsen, too, was unable to follow their course in sections of H. gordioides, or to detect the pores, though it is true his specimens do not appear to have been as fully mature as is one of my individuals.

There are two median unpaired sperm-sacs, or, more properly, septal pouches which act as sperm-sacs (figs. 1, 16).

Segment X is filled with loose masses of developing spermatozoa in all stages, mostly fully formed; the Septum X/XI is pushed backwards above the gut, and is also filled with sperms; the end of this sac is at about the level of the end of Segment XI. In Segment XI we have a repetition of this; its hinder wall is also pouched, and reaches to the middle of the thirteenth segment.

There is only a single pair of ovaries, which are situated in Segment XII; I sought in vain for a second pair both in the entire and in sectionised specimens.

A single pair of oviducts corresponding to these ovaries starts from large, wide, flat funnels in Segment XII (cf. figs. 1, 38). The oviduct (figs. 38—42) is a remarkably wide tube, of much greater diameter than the sperm-duct. It is at first directed backwards, and continues in this direction for some

distance; then it curves outwards and downwards towards the latero-ventral angle of the body-wall, which it penetrates well within the Segment XIII, to open just anterior and external to the ventral chæta. The pore is overlapped by a prominent flap, which seems to be entirely due to the greater development of the muscular coats of the body-wall in this segment (fig. 42). The position of this pore so far back in its segment is a very unusual one; for in nearly all the "limicoline" Oligochætes the pore is intersegmental, and even in the earthworms it is usually nearer the margin of the segment than it is in the present worm.

It should be stated that in the younger individual the testes and ovaries are quite small, and except for the rather larger nuclei in the female gonad and a more compact outline of the organ, there is no difference between the two sexes; yet in it the oviduct has already the character described for the adult—a comparatively wide tube (figs. 43, 45) with a wide funnel-shaped opening into the cœlom; the duct is traceable as far as the body-wall, which it reaches near to the ventral chætæ.

There is a striking difference both in dimension and in structure between the oviduct and sperm-duct, for whereas the latter has a very narrow lumen, which appears to be a perforation through a string of cells and is in many respects like a nephridium, the oviduct is quite a wide tube, surrounded by an epithelium of several cells, or, at any rate, a multinuclear syncytium, bearing long cilia within (figs. 44, 46).

The oviducal funnel does not project much into the segment, and in the younger individual has an appearance quite different from that presented by the young sperm-funnels, which are merely smaller representatives of the adult condition. The oviducal funnel, however, is here but little defined (fig. 45); the duct appears in longitudinal section as if the septum were pouched backwards to form a tube, which tube is lined by cells bearing cilia. The lip of the funnel, however, is ill defined; its upper margin is distinct enough and

formed of cubical cells, in which I could not detect cilia, but the lower lip is as yet not prominent; but by the time the worm is sexually mature the lip of the funnel becomes a much more prominent structure.

The hinder wall of Segment XII is pouched, and in the ovisac so formed are some large ova; others lie free in the segment, and still others are free in Segment XIII under the sperm-sac; while in the fourteenth segment still larger eggs distend the body (fig. 1). The presence of eggs in various stages of development in Segment XIII led me to expect a second pair of ovaries here, but I have failed to make them out. It is true that a small group of cells appears in transverse sections to be attached to the underside of the ovisac; this I took at first for a second ovary, but following the sections along, it became evident that it was only a group of small "nutritive" cells adherent to a larger ovum. The mass is free in the segment, and moreover there is no trace of a second pair of oviducts nor their funnels in either of my two specimens.

The funnel of the oviduct (in Segment XII) is so conspicuous an object, its nuclei are so deeply stained, and the funnel is so thick, that I feel sure that I have made no error in this matter. Moreover, in the longitudinal sections the three pairs of young gonads and funnels are quite evident, but no corresponding fourth pair exists.

In Segments XI, XII, and XIII there is a pair of solid glands connected with the epidermis. In the twelfth segment the gland opens in the neighbourhood of the ventral chæta on each side, but in each of the eleventh and thirteenth segments the two glands open below the nerve-cord in the median line. Each gland (fig. 30) consists of a group of long club-shaped cells, with faintly granular and vacuolated contents, which are not stained by hæmalum. The gland projects freely into the cœlom, and the necks of the cells are easily traceable through the epidermis. In each case the gland is nearly of the same length as the segment.

These "copulatory glands" are comparable to the glands of several Enchytræids.[1]

There are two pairs of globular spermathecæ (fig. 1) filled with spermatozoa, communicating with the exterior along the lateral line. They practically fill the anterior half of Segments VIII and IX; there is no differentiated duct, but the epidermis is here invaginated to pass through the muscles and reach the sac. The short tube thus formed is lined by cuticle; there are no special muscles around this tube.

Dimensions.—About 20 mm. by $\frac{1}{3}$ mm; about sixty segments. (The worm was not measured before it was cut in pieces for sectionising, but the portion cut longitudinally measures 10 mm., contains twenty-three segments; and the uncut remains measures 8 mm., contains thirty-one segments; while the transverse series of sections involves two [?] segments.)

Locality.—Lake Wakatipu, South Island, New Zealand, from a depth of 550 feet.

Remarks.

The new worm which I place in the genus Haplotaxis differs from the other two species in a number of minor points, but most noticeably in the possession of a single pair of ovaries and oviducts. The presence of a second pair of these organs has hitherto been a character of the genus which therein differs from all other Oligochætes except the Lumbriculidæ. But apart from the absence of the second pair of female organs, the new worm agrees in all other points with the generic characters as given by Michaelsen in his article in the 'Tierreich,' in the more detailed papers by Beddard, and in his Monograph. The possession of two pairs of sperm-ducts opening independently is another character of the genus, which, however, is shared by Pelodrilus. The latter genus was founded by Beddard (3) for a

[1] Forbes describes a pair of glands, of similar character apparently, in every segment of the body, and suggests that they are sensory.

worm from New Zealand (P. violaceus), in which the sperm-ducts present the peculiarity of both opening independently, but in the same segment. Since this genus is provided with only a single pair of ovaries, I have kept in view the possibility of this being the case in the new worm, but although I did not succeed in tracing the second pair of male ducts to the body-wall, yet there is nothing in the direction of the ducts to indicate that the first pair passes through an entire segment. Moreover, a second species of this genus, P. ignatovi, has recently been described by Dr. Michaelsen (6), in which the arrangement of the sperm-ducts is similar to that in Haplotaxis, so that the general arrangement of the genital ducts and pores in this species agrees pretty well with that described in H. heterogyne. But the agreement ceases here, for in all those anatomical characters by which Pelodrilus is distinguished from Haplotaxis the new species now under discussion agrees precisely with the latter. It forms, in fact, with P. ignatovi, a link between the genera Pelodrilus and Haplotaxis as originally characterised. This is seen in the following tabular summary of the characters under discussion, though there are several other differences between the two genera :

	H. gordioides.	H. smithi.	H. heterogyne.	P. ignatovi.	P. violaceus
Chætæ	4 isolated, dors. < vent.	4 couples, dors. > vent.	4 isolated, dors. < vent.	4 isolated, alike	4 couples, alike
Male pores	XI, XII	XI, XII	XI, XII	XI, XII	2 pairs on XII
Female pores	XII/XIII, XIII/XIV	XIII, XIV	XIII	XII/XIII	XII/XIII
Spermathecæ	7, 8, 9	7, 8	8, 9	8, 9	8
Sperm-sacs	Median	Median	Median	Paired; testes free	Paired; testes enclosed
Ovisacs	Median	Median	Median	Median	?

Nephridia and Genital Ducts.

From the point of view of general morphology, this new species of Haplotaxis is of considerable interest owing to the remarkable structural similarity that exists between the sperm-duct and the nephridium. The genus belongs to that section of the Oligochæta which in former days were termed "Limicoline" or Microdrili (mihi), in which excretory segmental organs are in the mature worm absent from the segments containing the genital ducts. This distinction is no longer of so much importance now-a-days, since Vejdovsky (**15**) has shown that in several families, viz. the Chætogastridæ, Naididæ, Enchytræidæ, Tubificidæ, and Lumbriculidæ, these nephridia are present in the genital segments of the immature worm, but disappear by degeneration before the genital ducts make their appearance; and Forbes (**4**) states that in H. emissarius (=H. gordioides) the anterior nephridia in Segments X to XV are small and rudimentary. Now the questions that naturally arise in connection with Haplotaxis heterogyne are: (1) Do nephridia exist in the immature worm in Segments X, XI? If so, then (2) have they disappeared in these segments and been replaced by the sperm-ducts, which have assumed the structure of nephridia? Or, on the other hand (3) have the nephridia persisted in these two segments and been converted functionally into the sperm-ducts? As we have no knowledge of the developmental history of any species of Haplotaxis, we cannot give a direct or certain answer to either of these questions, but the striking similarity between the two categories of organs presented by this species make it scarcely probable that the sperm-ducts have assumed the structure of nephridia, and renders it much more probable that the nephridia have been converted into sperm-ducts, the minute anatomy of which is so absolutely unlike that presented by these organs in other Oligochætes. The small degree of structural difference between the two organs in the present worm may be due to the difference in function. If this third question be answered

in the affirmative; if, that is to say, the nephridia in this worm do act in these two segments as sperm-ducts, then the question as to the homology of these ducts with nephridia in the class is to some degree reopened.[1]

I limit myself to the sperm-ducts, for there is no resemblance between the oviduct and the nephridium, and there need be no debate as to the homology between these. For it does not necessarily follow that if the sperm-duct be shown to be homologous with the nephridium, the oviduct would also be homologous; in point of fact, Vejdovsky (loc. cit., p. 158) expressly states that "there is not a complete homology between the oviducts and the sperm-ducts." And further, it is worthy of note that Bürger (**10**), in a recent paper on the development of Clepsine, finds considerable difference in the mode of development of the male and female organs in the Hirudinea. He shows that in the case of the female organs the entire apparatus, both gonads and ducts, is derived from a V-shaped "anlage"; whereas only the terminal portion of the male duct is derived from a corresponding V-shaped "anlage" in its segment, while the testes, vasa efferentia and v. deferentia develop from quite independent groups of cells, which are not represented in the female system. We may therefore, without prejudice to the larger question, confine ourselves for the moment to the sperm-duct.

It is unnecessary to recapitulate in detail all the points of resemblance and the few points of difference exhibited by the sperm-duct and the nephridium in the Oligochæta in general, or to repeat the historical arguments and views of Claparède and of Lankester in support of the homology; for this has been recently given by Beddard in his account of the development of Octochætus multiporus (**7**). It is sufficient to note that many modern zoologists have withdrawn their adherence to the theory involving any such homology, owing to the facts recorded in recent embryological memoirs; while the whole subject of "nephridium"

[1] See postscript, p. 322.

and "cœlomo-ducts" involved in the more recent theory has been summarised and reviewed by Goodrich (**11**), and has been accepted, and the ideas of terminology in connection with this view have been extended, by Lankester in his 'Treatise on Zoology' (part ii, p. 32).

According to this modern view, a sharp distinction, founded on the different modes of origin, is drawn between the excretory organs and the genital ducts of the Oligochæta. The former being, according to the observations of Vejdovsky (**16**) and of Wilson (**17**), derived from epiblastic ingrowths, the latter from mesoblastic outgrowths from the wall of the cœlom. There is still some doubt, however, as to whether the whole nephridium is epiblastic, for whereas Vejdovsky and Wilson derive it from a "nephric cord" of cells which originate from a superficial teloblast, Bergh (**9**) insists that the whole organ is developed from the funnel-cell, which he regards as mesoblastic in origin, and not as having pushed its way from the surface into the mesoblast. If this statement of Bergh's should turn out to be true—and it is a case of one good observer against two good observers,—it is clear that a modification will have to be made in the view as to the sharp distinction between the two categories of organs. However this may be, it seems clearly and satisfactorily determined that the genital funnel at any rate is formed as a proliferation of the cœlomic epithelium covering the anterior face of the septum to which the nephridial funnel is attached. Now, Goodrich (**12**) has shown in a series of valuable memoirs that in the Polychæta the "cœlomic funnel" or "cœlomostome," which functions as a genital funnel, may become grafted on to a nephridium, with or without the loss of the "solenocytes" of the latter organ.

It appears to me that in Haplotaxis heterogyne something of this kind has occurred, for the sperm-funnel is anatomically quite different from the nephridial funnel of the neighbouring segments; while the sperm-duct is pratically indistinguishable from a nephridial tube, and it originates from the funnel at the extreme ventral margin, in the posi-

tion, that is, in which a nephridial funnel, if it were present, would lie; in other words, the duct does not issue from the centre of the funnel as in the sperm-ducts of other Oligochætes. And I suggest that in this worm we have such a composite organ as Goodrich has described in several of the Polychætes (e. g. Goniada, Phyllodocids, Syllids), and to which Lankester gives the name "nephromixium."

In connection with the mode of origin of the sperm-duct from its cœlomostome, it is rather remarkable how little we really know; and it is as well to insist upon this absence of knowledge, and to note precisely how far embryologists have traced (a) the development of the genital funnel, and (b) the development of the duct from this funnel.

Vejdovsky (**15**) has put on record the general course of the history for Chætogaster, and in less detail for certain other genera in which, he says, the same course is followed. The genital funnel appears as a thickening of the peritoneal cells on the anterior face of the septum, and the genital duct grows back from it as a solid cord of cells; this cord reaches the epidermis and becomes hollowed out to form a tube.

In Chætogaster the nephridium has no funnel; but in Stylaria lacustris, which he proceeds to describe (p. 129), he finds that, first of all the nephridium of this segment, VI, gradually undergoes a retrogressive metamorphosis, breaking up into cells, which separate till nothing but the nephridial funnel remains on the anterior face of the Septum V/VI. This, he says, persists for a long time. His next stage figured represents the flat, thick, genital funnel in place of the small nephridial funnel. He does not state in so many words that the latter goes entirely, and it is possible, in the light of Bergh's researches, that it may contribute to the formation of the genital funnel.

At any rate, there is apparently no doubt, in spite of what Roule later on suggests, that a nephridium lies at first in the segment, then disappears; that the genital funnel is formed from the cœlomic epithelium, and gives rise to the genital duct.

Bergh (**8**), in 1886, describes in detail the development of the funnels in Lumbricus, but he failed to trace the development of the genital ducts therefrom. The genital funnel develops as a thickening of the peritoneal cells immediately above (dorsad of) the nephridial funnel (which of course does not disappear in this earthworm); the development involves partly the cells forming a covering for the back of the nephridial funnel and partly the cells forming the anterior face of the septum itself. The same is true both for oviduct and sperm-duct, and this very close association of nephridial funnel and cœlomostome is, it seems to me, likely to be of interest when the development of the entire nephridial funnel is fully known.

At present we do not know whether the "marginal" cells of the Lumbricid nephridial funnel are cœlomic in origin. It is quite possible that they are. For the "central cell" is probably the original funnel-cell, which, according to Vejdovsky, divides so as to permit the tubule to communicate with the cœlom. If it should turn out that these peripheral marginal-cells are cœlomic, then the close topographical relation of genital funnel with the nephridial funnel described and figured by Bergh will indicate that the whole "nephridium" of earthworms is a "nephromixium."

A wholly different history is given by Roule (**13**), in 1889, for an Enchytræid. In the earlier stages in the development of "Enchytræoides marioni" the twelfth segment, in which at a later period the sperm-ducts will arise, contains no excretory organs, though these are present in the preceding segments, IX, X, XI, and in the segments following it, namely, XIII, XIV, etc. The young sperm-duct, when it does ultimately make its appearance, is "en tout semblable à une très jeune néphridie, et deplus, il occupe exactement la place qu'aurait l'organe segmentaire s'il s'était developpé dans la XIIme anneau." Its mode of origin is stated to be quite like that of a nephridium, after this has separated itself from the nephric cord (which is observable in the posterior, but not in the anterior segments); it now consists of

three or four cells more or less fused to form a syncytium; and Roule considers it certain that the sperm-duct is nothing else than the nephridium of this twelfth segment, which is late in appearing, for its special function is not called into play till a much later stage than that of the segmental organ.

It will be noted that this is a very different history from that given by Vejdovsky; and it is well to note that Roule studied sections through successive stages, whereas it appears from Vejdovsky's words that he studied living specimens and entire preparations only.

Then follows Beddard (**7**), who dealt with a "micronephric" earthworm, Octochætus multiporus, a form in which the earliest segmental organ is a "meganephridium," which becomes broken up into a number of micro-nephridia which are without funnels. According to this author the original funnel of the meganephridium persists in the genital segments, and becomes converted into the genital funnel in each case; whilst the genital duct is for the first part of its course derived from part of the nephridium, which starts to grow once more, and extends back to form the rest of the genital duct (p. 578).

If, now, we consider these various statements, and if we regard, as I believe most zoologists will do, the "Limicoline" Oligochætes as ancestral to the "Terricoline," it seems probable that, phylogenetically, the history of affairs with regard to the organ under discussion has been somewhat as follows:

First stage.—The nephridia act as genital ducts, for Stolc (**14**) finds that in Aeolosoma spermatozoa may escape from all or any of the segmental organs; in the genital segments these are slightly larger than in other segments, though otherwise similar to them. The funnel of the nephridium is of very simple structure, and there are no representatives of the "marginal cells." Further, according to Roule, the sperm-duct is a late-appearing nephridium in one species of Enchytræid.

Second stage.—A special cœlomostome becomes developed, which, added to the nephridium, increases the efficiency

of the organ as a collector of spermatozoa; we thus have a "nephromixium" comparable to the arrangement in several genera of Polychætes. Such is the condition of the sperm-duct in Haplotaxis heterogyne, as I believe; while in Octochætus multiporus a short part of the genital duct is apparently also purely nephridial in origin.

Third stage.—The cœlomostome gives rise to its own cœlomo-duct, which may either coexist in the genital segment with the nephridium (as in most "terricoline" Oligochætes), or the nephridium, owing perhaps in some cases to the small size of the worm, disappears from the segment during or before the development of the genital duct (as in "limicoline" Oligochætes and Pontrodrilus).

We have, then, to some extent a parallel series of phenomena analogous to those described with so much care by Goodrich in the Polychæta, from which it would appear that the sperm-ducts are not absolutely homologous throughout the Oligochæta.

Bibliography.

A. Haplotaxis, etc.

1. Beddard, F. E.—'Ann. Mag. Nat. Hist.,' 1888 (ser. 6), vol. i, p. 389.
2. Beddard, F. E.—'Trans. Roy. Soc. Edinb.,' 1889, vol. xxxv, p. 629.
3. Beddard, F. E.—'Trans. Roy. Soc. Edinb.,' 1890, vol. xxxvi, p. 292 (Pelodrilus violaceus).
4. Forbes, S. A.—'Bullet. Illinois State Lab. Nat. Hist.,' vol. iii, p. 107.
5. Michaelsen, W.—'Zool. Jahrb. (Syst.),' 1898, vol. xii, p. 105 (synonymy of Phreoryctes).
6. Michaelsen, W.—'Verh. Naturw. Verein. Hamb.,' 1903 (P. ignatovi).

B. Remarks on Genital Ducts.

7. Beddard.—'Quart. Journ. Micr. Sci.,' vol. xxxiii, p. 522.
8. Bergh.—'Zeit. f. wiss. Zool.,' vol. xliv, p. 303.
9. Bergh.—'Zeit. f. wiss. Zool.,' vol. l, p. 498.
10. Bürger.—'Zeit. f. wiss. Zool.,' vol. lxvii, p. 534.
11. Goodrich.—'Quart. Journ. Micr. Sci.,' vol. xxxvii, p. 477.

12. Goodrich.—'Quart. Journ. Micr. Sci.,' vols. xl, xli, xliii.

13. Roule.—'Ann. Sci. Nat.' (Zool.), ser. 7, vol. vii.

14. Stolc.—'G. B. Böhm. Gesel.,' 1889 (abstracted by Beddard, loc. cit. supra).

15. Vejdovsky.—'Syst. u. Morph. Oligochæt.'

16. Vejdovsky.—'Entwick. Untersuch.'

17. Wilson.—'Journ. Morph.,' vol. iii, p. 419.

EXPLANATION OF PLATES 16—18,

Illustrating Dr. W. Blaxland Benham's paper, "On a New Species of the Genus Haplotaxis; with Remarks on the Genital Ducts of the Oligochæta."

Reference Letters.

br. Cerebral ganglion. *b. w.* Body-wall. *c. c.* Cœlomic corpuscles. *c. ep.* Cœlomic epithelium. *c. m.* Circular muscles. *cu.* Cuticle. *d.* Dorsal chæta. *d. v.* Dorsal blood-vessel. *ep.* Epidermis or epithelium. *g.* Gizzard. *gl.* Copulatory gland or its external opening. *int.* Intestine. *l.* Lateral line. *l. m.* Longitudinal muscles of body-wall or other organ. *m.* Muscle-fibres. *m. ch.* Muscles of chæta. *n.* Nucleus. *n. c.* Ventral nerve-cord. *ne.* Nephridium. *ne. d.* Nephridial duct. *ne. o.* Position of nephridiopore. *n. f.* Nephridial funnel. *o.* Ovum. *o. d.* Oviduct. *œ.* Œsophagus. *o. f.* Oviducal funnel. *ov.* Ovary. *ov. s.* Ovisac. *p.* Lateral prominence outside ovipore. *s.* Septum. *s. c.* Circle of sensory cells surrounding a segment. *sp.* Spermatozoa. *sp. d.* Sperm-duct. *sp. f.* Spermiducal funnel. *sp. s.* Sperm-sac. *spth.* Spermatheca or its aperture. *t.* Testis. *v.* Ventral chæta. *v. v.* Ventral blood-vessel. ♂ Male pore. ♀ Female pore.

PLATE 16.

Fig. 1.—View of the anterior extremity of a mature specimen of Haplotaxis heterogyne stained and mounted in Canada balsam. (Camera, × 40.) In the anterior segments the circular segmental series of sensory cells are shown; further back these are indicated in the optical section of the body-wall. The extreme dilatation of Segments XIII and XIV is partly due to compression. The small size of the intestine in the genital segments is shown. *o. d.* is the funnel of the oviduct.

Fig. 2.—A transverse section across the gizzard. (Camera, × 500.) The

greater part of the wall consists of circular muscles, between which, towards the external surface, bundles of longitudinal muscles are intercalated.

Figs. 3—7 represent five sections of one of the post-ovarian nephridia, as seen in transverse sections of the immature individual. These five are selected out of nineteen sections which involve a single nephridium. (Camera, × 120.)

Fig. 3 shows the nephridial funnel projecting through a septum, and a small part of the post-septal region of the nephridium.

Fig. 4 is at about the middle of the series, showing the nephridium at its greatest height.

Fig. 5 involves the muscles of the ventral chæta; the nephridium is passing downwards towards the body-wall.

Fig. 6 is a few sections onwards.

Fig. 7 shows the short "duct," represented by more granular cells (see fig. 10).

Fig. 8.—An enlarged view of a nephridium in such a section as is represented in fig. 4. (Camera, × 500; details as seen with Leitz, $\frac{1}{12}$ homog. imm. lens.) The vacuolated condition of the large nephridial cells, and the distinct "wall" to the canal are seen.

Fig. 9.—A funnel of a post-ovarian nephridium from a longitudinal section, which is neither sagittal nor frontal, but which cuts the worm obliquely. (Camera, × 700.) No details of cell-structure are shown.

Figs. 10, 11.—Two consecutive sections through the "duct" of the nephridium. (Camera, × 700; details under $\frac{1}{12}$ hom. imm.) The cytoplasm of the nephridial cells, or better "syncytium," is no longer vacuolated, but highly granular.

Fig. 10 is a highly magnified view of fig. 7.

Fig. 11 is the next section. It shows the "duct" passing into and through the epidermis, from which it is readily distinguished. I was unable to trace the canal to a pore.

Fig. 12.—A transverse section through the body of the mature individual, nvolving the first nephridium in Segment X. (Camera, × 120.)

Fig. 13.—The same nephridium—next section—more highly magnified. (Camera, × 700.) The cytoplasmic network not indicated. Towards the upper part of the organ the wall of the canal (*a*.) is much thicker than elsewhere.

Fig. 14.—The funnel of the first nephridium, as seen in a longitudinal section of the immature individual. It is smaller than that of the post-ovarian funnel (cf. fig. 9). (Camera, × 700.)

Fig. 15.—A transverse section of the body through the œsophageal region (× 120), showing the relative sizes of the dorsal and ventral chætæ.

PLATE 17.

FIGS. 16—24 represent a series of nearly consecutive transverse sections of the mature specimen through the first pair of sperm-funnels and the second pair of testes. (Camera, × 120.)

In fig. 16 the entire section, with all the organs involved, is drawn; in the rest only the ventral half or less is drawn. In fig. 16 the spermatozoa filling the first sperm-sac and surrounding the gut are shown, but they are omitted in subsequent figures. The worm having been first mounted entire and somewhat compressed, the organs have been slightly displaced, so that the right and left organs are cut through at different planes in a section. On the right side the course of the sperm-duct can be followed easily up to fig. 20, where it has reached its greatest height in the body; it then descends, and the last trace that I was able to detect (fig. 22) was close to the chætal muscles; the base of the chæta is cut through here, but the shaft comes into view and perforates the body-wall nine sections further along. The body-cavity was here filled with a coagulum, which, being stained in hæmalum, rendered it impossible to trace the sperm-duct further; but on the left side the duct was traced right up to the epidermis (fig. 24) (see also fig. 29).

FIGS. 25—27.—Three consecutive sections through part of the left sperm-duct in the region shown in figs. 20, 21, in order to show the structure of the duct and the presence of spermatozoa therein. The sperms (*sp.*) can be traced in several other sections, even when the duct is close to the body-wall (cf. fig. 29). (Camera, $\frac{1}{12}$ hom. imm., Leitz; × oc. 3, Leitz.)

FIG. 28.—A section cutting the sperm-duct (of Segment XII) longitudinally near the upper end of its course, showing the upward and downward limbs of the duct. (Camera, × 700.)

FIG. 29.—From a transverse section (fig. 24), showing the sperm-duct passing through the muscles of the body-wall towards the epidermis, which is reached in the next section (not figured). Spermatozoa are seen in one of the sections across the duct. (Camera, × 700.)

FIG. 30.—The copulatory gland from Segment XIII, as seen in a transverse section of the mature worm. (Camera, × 700; details under $\frac{1}{12}$ hom. imm.)

PLATE 18.

FIGS. 31—36 show the sperm-funnel and the commencement of its duct. Camera, × 700; details under $\frac{1}{12}$ hom. imm.)

FIGS. 31—34 are four consecutive sections through the first sperm-funnel on the right side.

Fig. 31 cuts through the lip of the funnel.

Fig. 32 cuts through the middle of the funnel; it shows the sperm-duct issuing from the extreme ventral margin, and a few spermatozoa, with which the segment is filled, are seen entering the mouth of the duct.

Fig. 33 cuts across the sperm-duct as it bends backwards behind the funnel; a spermatozoa is seen in the duct as a small dot. (This figure is an enlargement of fig. 16.)

Fig. 34, which is from a section between those drawn in figs. 16 and 17, involves the lip of the funnel and the root of the second testis, below which is the sperm-duct.

Fig. 35 is an enlarged view of a section near that represented in fig. 18. The funnel is no longer present; the second testis is seen, and the sperm-duct is cut through below the testes, and again on the right of the figure.

Fig. 36, from a section intervening between those represented in figs. 19 and 20, shows the sperm-duct passing upwards behind the septum. The ciliation of the duct is shown in this figure.

Fig. 37.—A longitudinal section through the second sperm-funnel and duct and the ovary of the immature specimen. (Camera, × 700.)

Figs. 38—42 represent a series of successive but not consecutive transverse sections showing the oviduct. (Camera, × 120.)

In fig. 38 all the organs in the left half of the section are shown; in the remainder only the organ in question. In this section the oviducal funnel is cut through at about its widest part, but somewhat obliquely.

Fig. 39 (which represents the fourth section after the previous one) cuts through the lower part of the funnel, which was torn in the section (cf. fig. 44). In this figure half of the copulatory gland is seen (cf. fig. 30).

Fig. 40 (the fourth section beyond the previous one) cuts the oviduct somewhere about the middle of its course.

Fig. 41 represents the eighth section from the last, shows the duct entering the body-wall, which is here and in the next few sections much thicker than elsewhere.

Fig. 42 is the third from the preceding; the duct is now close to the epidermis. In the following section (not figured) the duct opens to the exterior below the prominence (*p.*), due to the greater development of the longitudinal muscles of the body-wall.

Fig. 43.—The oviduct (in longitudinal section) of the immature specimen. (Camera, × 120.) It shows practically its full length, and it will be noticed that it reaches back as far as the chætal muscles.

Fig. 44.—A transverse section of the oviducal funnel (see fig. 39), the wall of which has been ruptured during manipulation. (Camera, × 700; details under the $\frac{1}{12}$ hom. imm.)

Fig. 45.—Enlarged view of fig. 43, combined from it and neighbouring

section. The lip of the funnel is only definitely formed on its dorsal border, where it is seen passing upwards in front of the septum; on this lip I could detect no cilia, though these are quite evident in the duct itself. The septum is seen to be somewhat pouched backwards. (Camera, × 700.)

Fig. 16.—A transverse section of the oviduct about mid-way between figs. 40 and 41. (Camera, × 700.)

Postscript.—Since despatching my MS. from New Zealand I have come across an article by Mr. Beddard in the 'Proc. Zool. Soc.' in 1902, vol. ii, p. 89, which I had unfortunately overlooked. In discussing the female reproductive organs of Eudrilus he introduces some remarks, on p. 95, relative to "nephridia" and "cœlomo-ducts" which are in agreement with the views put forward in the present paper. He is "not convinced" that the oviducts and sperm-ducts are "cœlomo-ducts." Further, he makes use of Bergh's account of the origin of the genital duct from the "peritoneal" covering of the nephridial funnel in the same manner as I have done, and indicates the probability of part of the nephridial funnel being peritoneal in origin. He concludes (p. 97), "It appears to me that these various considerations show that it is at least premature to regard the gonad-funnel of the Oligochæta as essentially different from the nephridial funnels."

I regret that by this oversight my friend's views receive no recognition in the body of my paper.

June 6th, 1903.


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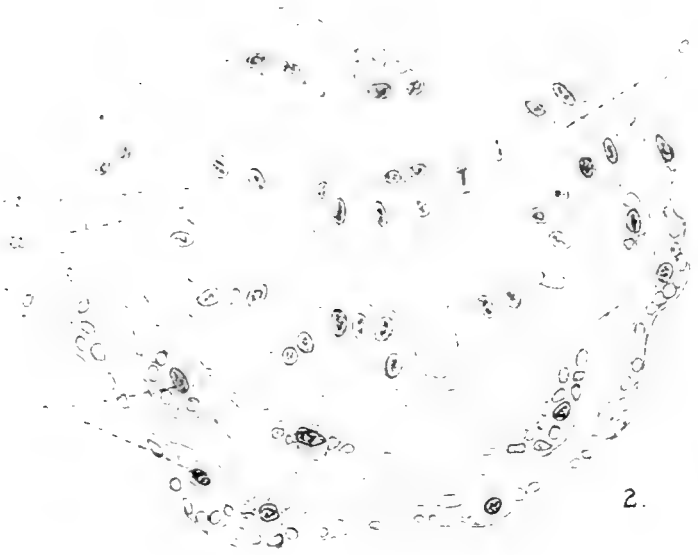
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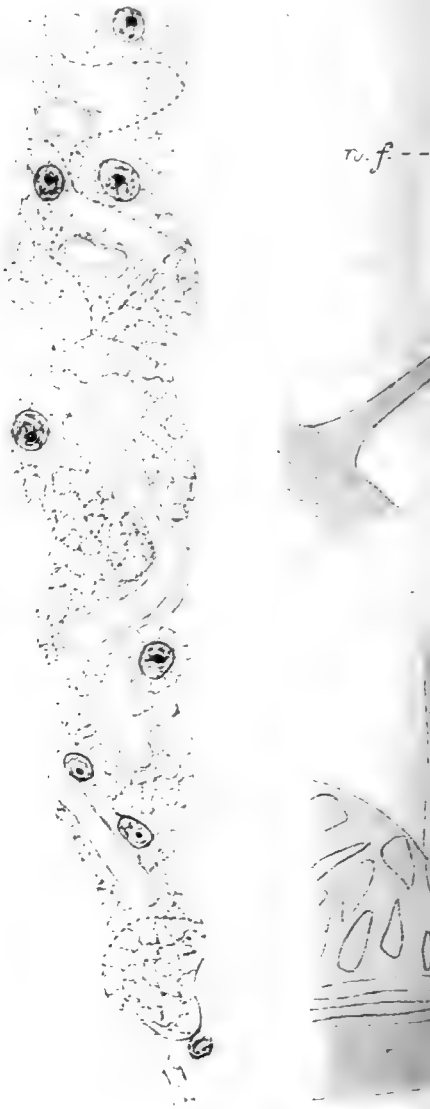
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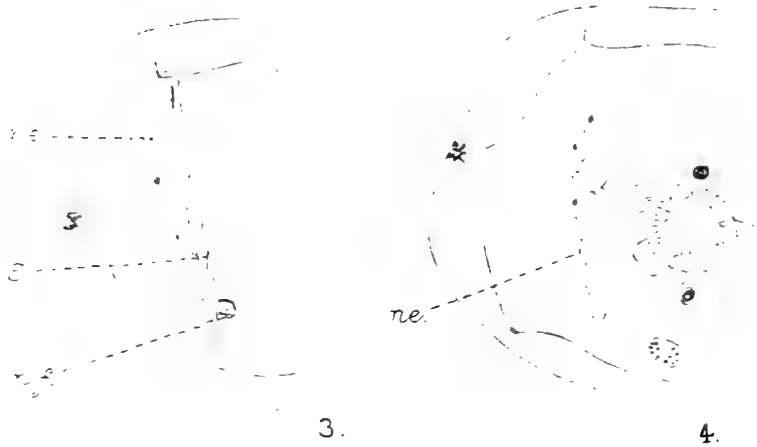
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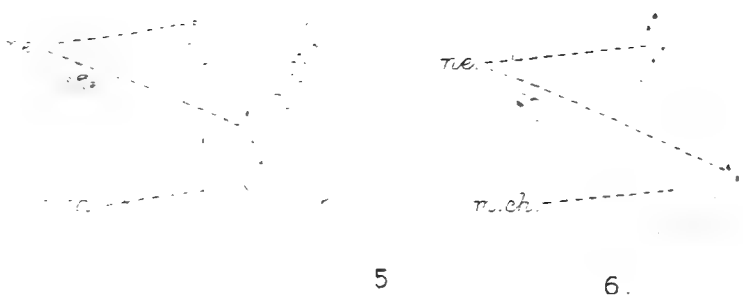
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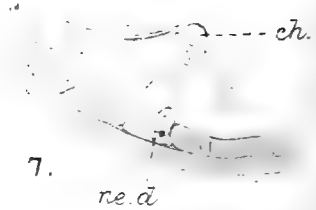
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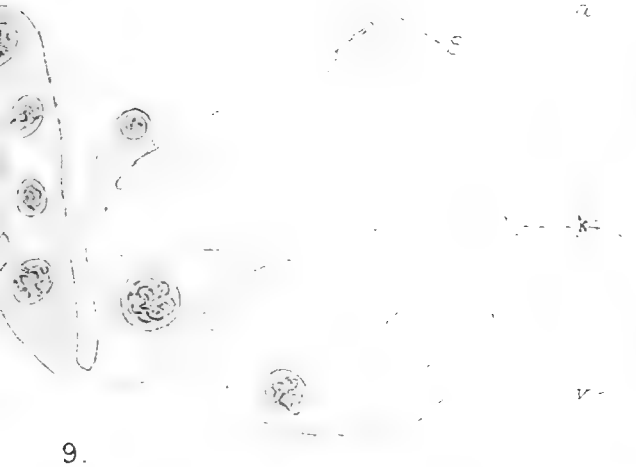

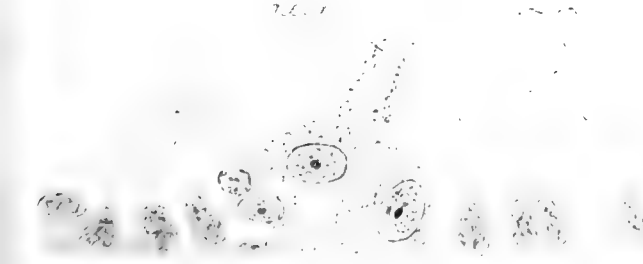

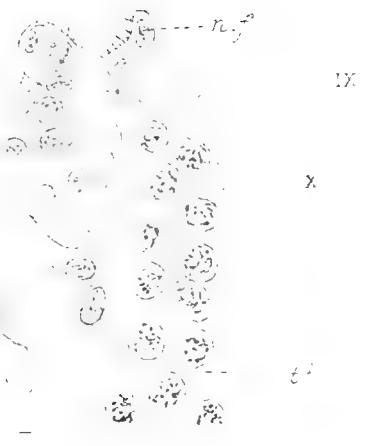

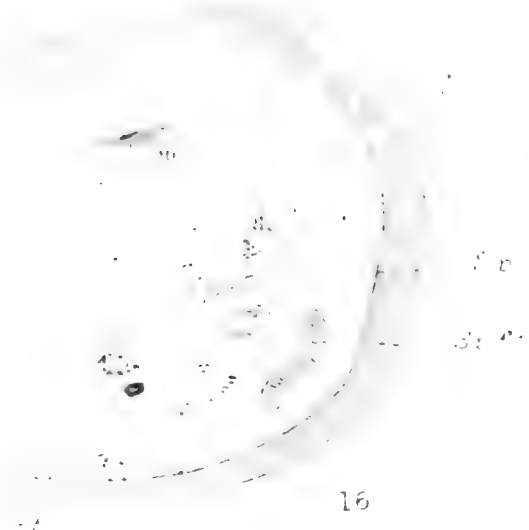

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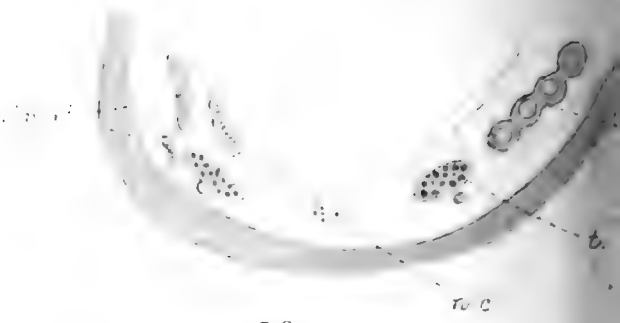

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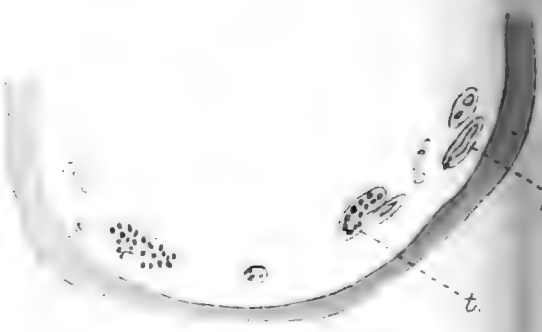

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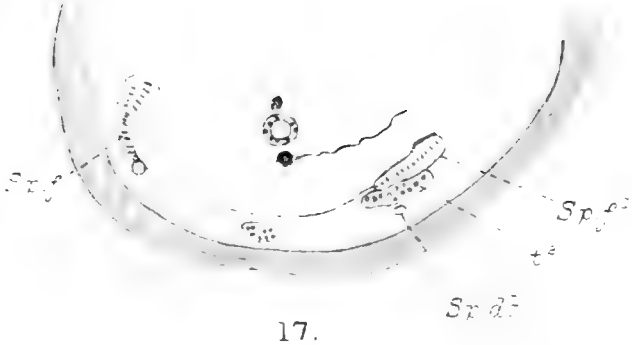

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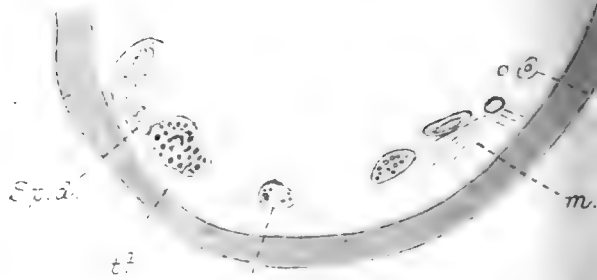

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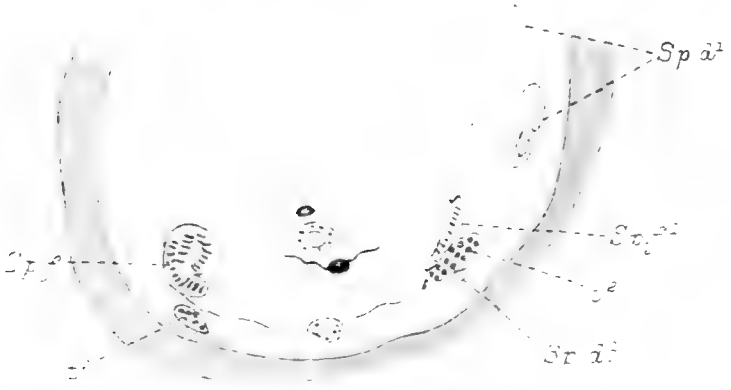

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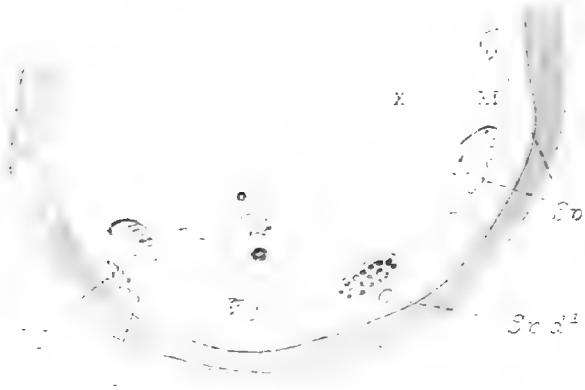

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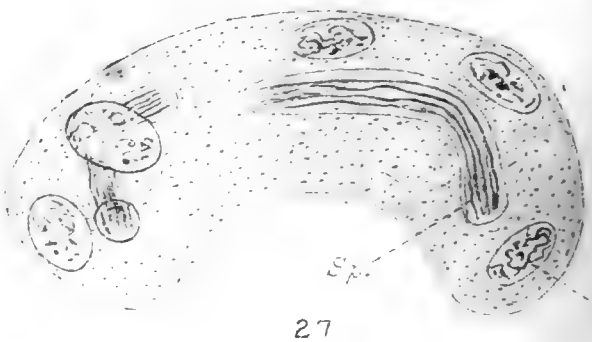

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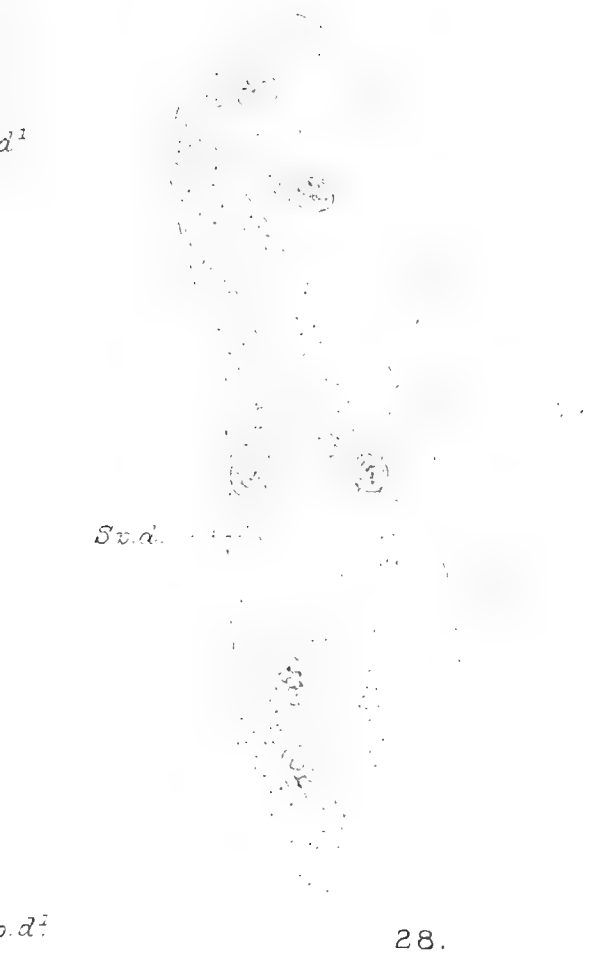

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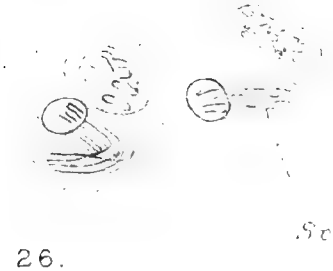

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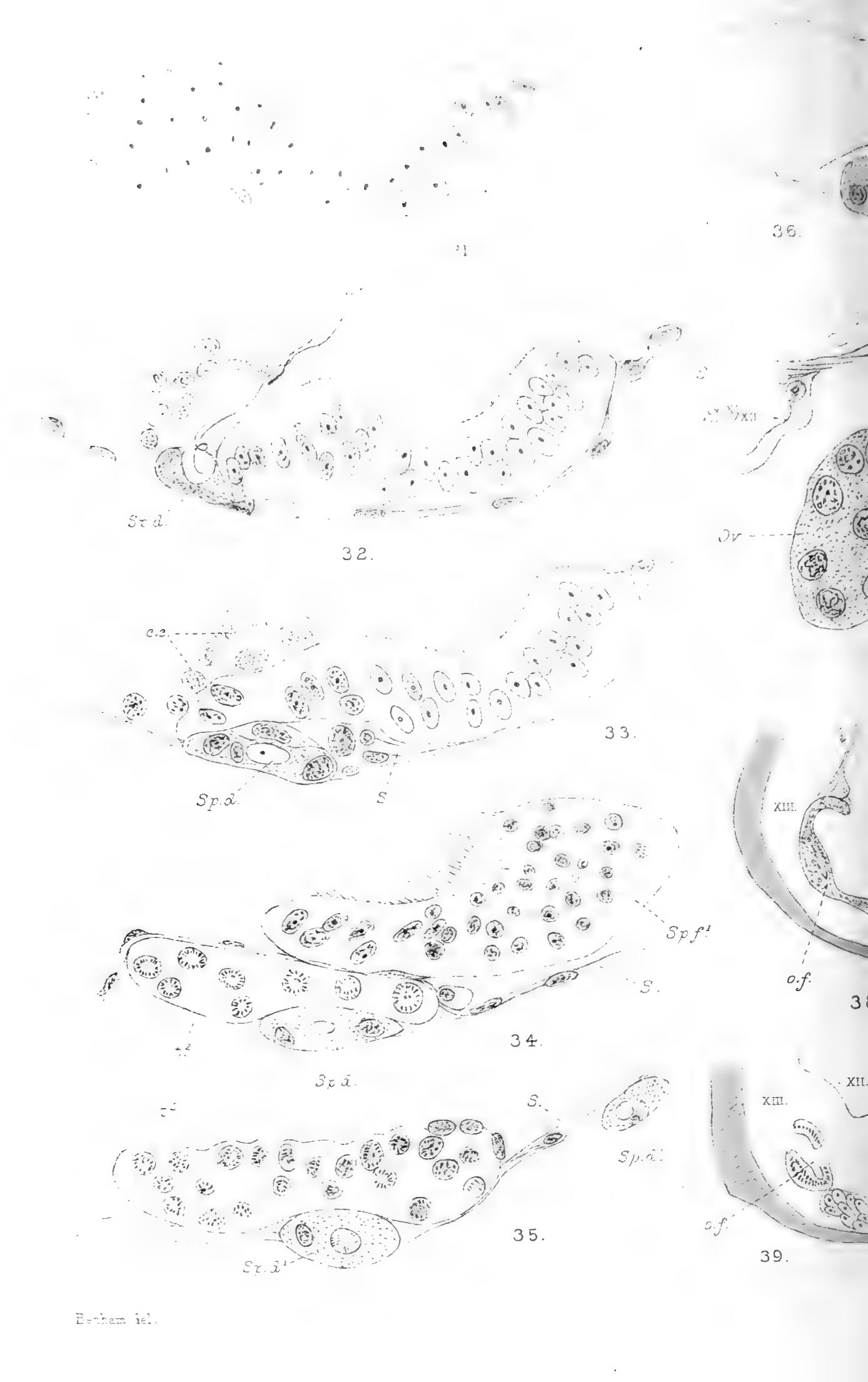

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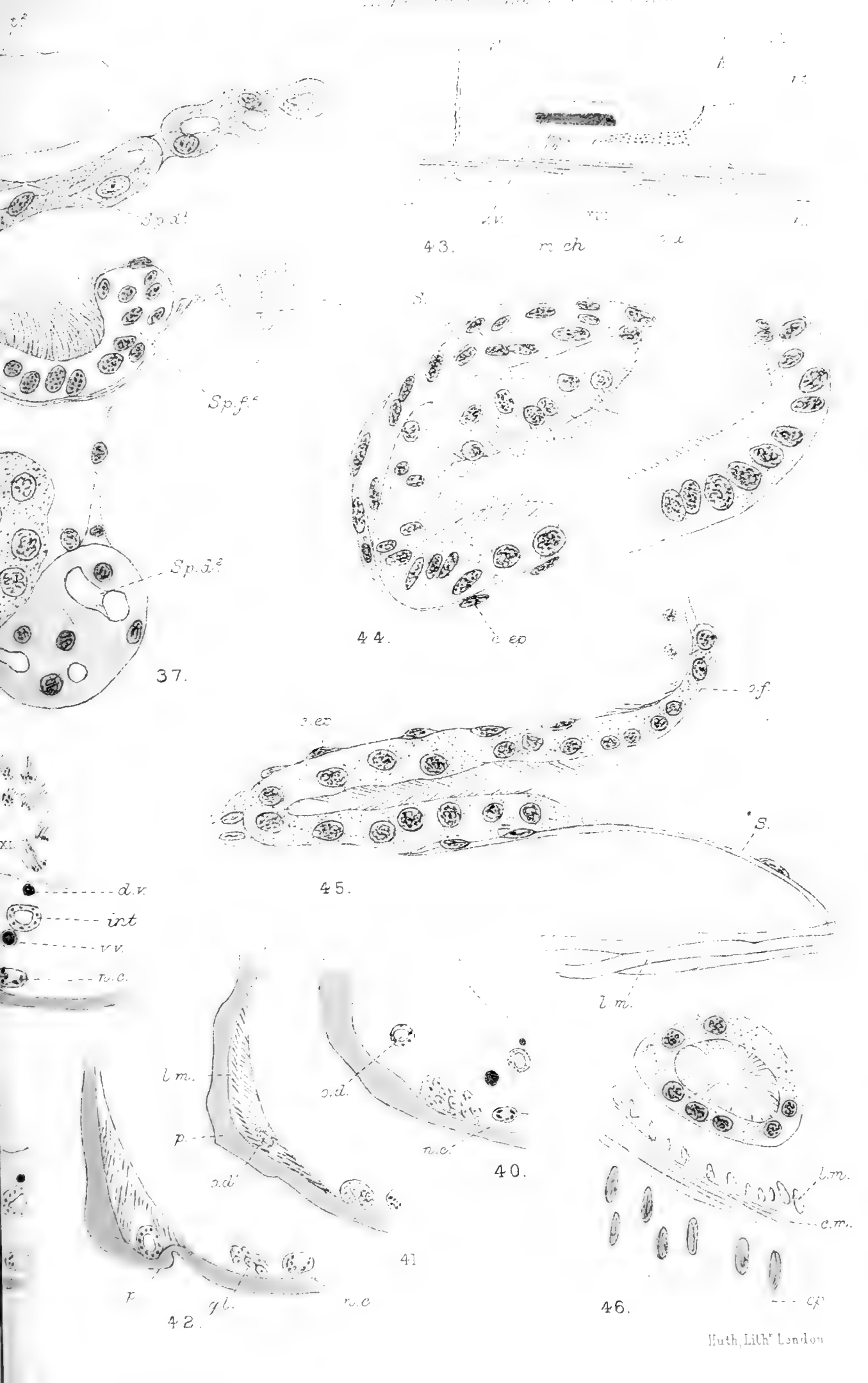

Huth, Lith^r London

The Œstrous Cycle in the Common Ferret.

By

Francis H. A. Marshall, D.Sc.

With Plates 19—21.

"Œstrus vocatur hoc malum."—Pliny.

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Introductory.

The investigations which form the subject of the present paper were commenced in the summer of 1901, and were carried on at the University of Edinburgh in connection with the Zoological Department. A preliminary account has already been published, being included in the memoir on 'The Œstrous Cycle and the Formation of the Corpus Luteum in the Sheep' (Marshall, 1903).

Through the courtesy of Professor Schäfer I was permitted to make use of the resources of the Physiological Department for keeping the ferrets used in the research. Both "polecat

ferrets" and white ferrets were employed, and were kept under constant observation.

The material for the histological part of the work was generally fixed and preserved in a 10 per cent. solution of formalin, and afterwards treated in the usual way for section cutting. Sometimes corrosive sublimate was used instead of formalin as a fixing agent. The stains ordinarily employed were a combination of hæmatoxylin and eosin.

I wish to record my obligations to Professor Ewart and Professor Schäfer for the encouragement and assistance which they have rendered me in furthering my researches. To Mr. Heape, also, I must express my indebtedness for valuable suggestions on a subject which he has made peculiarly his own. Lastly, I take this further opportunity of thanking Sir Thomas Gibson Carmichael, Bart., for his great generosity in providing an endowment.

The Œstrous Cycle.

The ferret is monœstrous, the female usually coming in season at the end of March or beginning of April. If permitted to become pregnant at this time a second sexual season may be entered upon in July, while occasionally ferrets have been known to breed three times within twelve months (Carnegie and other authorities, 1901).[1]

I do not know whether the female ferret ever experiences a second sexual season after failing to become pregnant during the first œstrus. It is frequently stated by fanciers that for ferrets to live healthily it is necessary for them to breed, and that "a doe ferret will sometimes die the first

[1] The above statements are based upon information given by ferret breeders (cf. Carnegie, etc., 1902). In my paper on the "Œstrous Cycle in the Sheep" (1903) I stated that the ferret was monœstrous and had a single sexual season annually. This conclusion, which is only sometimes correct, I had deduced from my own observations, having never had a ferret which experienced more than one œstrous cycle. As stated in the text, I have kept ferrets from October to the end of March, during which time they showed no signs of coming "on heat." A ferret fancier assures me that only very exceptionally has he known ferrets come in season between August and February.

time she is refused access to the buck" (Carnegie, etc., 1902). Several of my ferrets grew unhealthy and died during the sexual season, and while still "on heat," and I am disposed to believe that the mortality was partly due to their being refused copulation.

The period of œstrus in the absence of the male I have found to be extremely prolonged. In one individual it extended for six weeks, at the end of which time the animal was killed, the uterus being found to be in a condition of advanced recuperation. In another ferret, however, in which œstrus was observed in the beginning of June (at the time when it was procured), the period of "heat" was completely over at the end of the first week of July, coition not having been permitted. Five bitch ferrets which I obtained in the month of October lived perfectly healthily during an anœstrous period which extended until the close of the following March, when they began to show signs of coming "on heat," and were subsequently killed during the sexual season.

It appears then, that the ferret, to some extent, showed a transition between the monœstrous and polyœstrous condition, since in those individuals which experience two breeding seasons these are restricted to the spring and summer; so that it must be a matter of some doubt whether the time between the two "heat" periods should be correctly described as a diœstrous or an anœstrous interval. But, as already indicated, this interval is, as a matter of fact, generally, or perhaps always, occupied partly by gestation.

A number of interesting observations bearing on this subject have been made by Mr. A. H. Cocks, who has kept several members of the family Mustelidæ in activity. A female otter is described (Cocks, 1881) as coming in season nearly every month in the absence of the male. Upon a male being introduced, copulation was observed on July 17th, and a second time on August 12th, or nearly a month later. Young were born on October 12th, so that pregnancy lasted apparently for sixty-one days. From these observations it may be inferred that the female otter is polyœstrous in the

absence of the male, the duration of the diœstrous cycle being about a month, there being also a longer anœstrous period.

Bell (1874) describes the otter as having young in March or April, thus indicating that the wild otter has a single sexual season about the beginning of the year. The same author states that the progeny of the stoat are produced in April or May, while the polecat, of which the ferret is a domesticated variety, is said to give birth to young in May or June. These animals, therefore, are probably monœstrous, or perhaps diœstrous, while the weasel may perhaps be inferred to be polyœstrous from Bell's account (1874).

With the pine-marten, in captivity, it appears from Cocks' description (1900) that the œstrous period may extend to about a fortnight. A female was noticed to deposit here and there in her cage little mouthfuls of straw, an indication of her being in season, this habit having been previously observed in the case of the otter. A male was admitted on January 5th, shut off on the 16th, readmitted on the 17th, and finally separated on the 18th. Copulation is supposed to have occurred probably on the 8th, 10th, and 13th, and possibly also at other times, but was never actually observed. Young were produced on April 22nd. Cocks states that it is hazardous to allow the male and female to run together at other times than the œstrous period, as it is apt to result in the death of the female.

The badger is probably monœstrous, with an annual sexual season, its period of gestation being between four and five months (Meade-Waldo, 1894). (See postscript at end of paper.)

I made no observations on the length of the ferret's gestation, but this period is generally stated to be about six weeks, or approximately the same as that observed for the polecat (Harting, 1891; Cocks, 1891).

External Evidence of the Pro-œstrum and Œstrus.—The pro-œstrum with the female ferret appears to extend for about three weeks, and is characterised by a marked swelling of the vulva and a sanguineo-mucous flow. With

two or three individuals I did not observe any external bleeding, but it may have occurred and escaped my notice, since it was sometimes impracticable to make regular observations upon the animals during their prolonged sexual season. But bleeding into the uterine cavity, as I shall presently show, regularly occurs at the pro-œstrum, and is accompanied by a greater or less removal of uterine mucosa. I am inclined to think, however, that the discharge so formed is usually disposed of very gradually.

During the pro-œstrum, as at all other times during the cycle excepting at the œstrous period, the female will not permit copulation.

The period of œstrus can be recognised by the behaviour of the female ferret towards the male. The vulva remains enlarged, and a slight flow of mucus may continue to be discharged at the external genital aperture. As before remarked, the œstrus may last for several weeks, and is associated throughout with the swelling of the vulva. This extension, in the absence of pregnancy, of the period of desire, is perhaps comparable to what occurs in the case of bears in captivity, for with these animals in the Zoological Society's Gardens œstrus is said to last continuously for two or three months. (Heape, 1900.)

The female ferret, as already described, is monœstrous, coming in season about the end of March, but presents a transition to the polyœstrous condition in sometimes having a second (and occasionally a third) œstrous cycle in the summer months. In showing this tendency towards a concentration of sexual seasons the ferret may be regarded as standing midway between such animals as the dog or cat which are monœstrous, with, as a rule, two fairly regularly recurring œstrous cycles, and the otter, which, in captivity at any rate, is polyœstrous, and has a recurrent diœstrous cycle of a month's duration. (Cocks, 1881.)

So far as I am aware there is no periodicity of the sexual season with the male ferret, which is said to be capable of copulation at any time of the year.

Ovulation.

So far as my observations go, ovulation in the case of the ferret probably takes place at the beginning of the period of œstrus, but only as a result of coition. If the female is not allowed to copulate the mature follicles and contained ova appear to undergo atresia, notwithstanding the continuance of the œstrus. As a consequence the female fails to become pregnant if warded too late in the season. Thus the persistence of the œstrus, which may continue far into the recuperative period of the uterus, or even beyond it, is associated with degenerate follicles in the ovary. These facts may perhaps afford an explanation of the observations made by Robinson (1893), who found that, with the ferrets employed in his investigation, coition very frequently did not result in pregnancy, although the animals might have copulated more than once during œstrus.

The extension of the period of œstrus under conditions such as to preclude the possibility of the occurrence of pregnancy can only be regarded as one of those "disharmonies" in the apparatus of reproduction upon the existence of which in the animal and human organisation Metchnikoff in his recent work (1903) has laid so much stress.

A bitch ferret which I artificially inseminated failed to become pregnant, owing probably to the presence of the spermatozoa in the uterus without the additional stimulus of coition failing to induce ovulation; but it may have been in this case also that the mature Graafian follicles had begun to degenerate, and that the season for ovulation had passed by.

In failing to ovulate during œstrus except as a result of coition the ferret resembles the rabbit in some cases at any rate (Heape, 1897), and the sheep more exceptionally (Marshall, 1903). The majority of the mammalia in which the subject has been investigated have been found to ovulate spontaneously when "on heat."

Fig. 9 represents a section through an atretic follicle from a ferret in which œstrus had lasted for at least three weeks,

and perhaps longer. The animal had copulated on the day on which it was killed, but not previously during that œstrus. The ovum is seen to be much shrunken and obviously degenerate, while it is no longer surrounded by a discus proligerus. The membrana granulosa has almost completely disappeared, but a few cells in an advanced state of degeneration remain scattered in the cavity. There is the beginning of a loose ingrowth of connective tissue, but this, at the stage under consideration, is very slight. The connective-tissue wall of the follicle presents the appearance of being composed of very irregularly arranged strands, the distinction between theca externa and theca interna having become obliterated, while there is no distinct line of separation from the outlying ovarian stroma.

The Formation of the Corpus Luteum.—I made no attempt to obtain a series of stages illustrating the development of the corpus luteum in the ferret. Such few examples as I have examined show the usual ingrowth among the lutein cells of connective tissue from the follicle's wall; and, although, taken by themselves, they do not prove that the lutein cells are derived from the membrana granulosa, they are, in a general way, confirmatory of the description given elsewhere of the origin of the corpus luteum in the mouse, the rabbit, and the sheep, there being distinct evidence of the interepithelial nature of the ingrowth. I have also lately obtained sections through a young corpus luteum of a cat which, at the time of killing, was "on heat," or had been very shortly before; and these sections show the same point.

Since the publication of my account a paper by Cohn (1903) describing an experimental investigation on the mode of formation of the corpus luteum in the rabbit has appeared, and the result of this investigation has been to further confirm the view that the lutein cells are formed from the follicular epithelium. Cohn obtained a series of stages, the animals being killed at stated intervals after coition.

A similar conclusion has been arrived at by Sandes, who describes the process of formation of the corpus luteum of

Dasyurus in a paper read before the Linnean Society of New South Wales and abstracted in 'Nature' (1903). This author states further that the corpus luteum atreticum is formed in the same way as the corpus luteum verum, a result which, so far as I am aware, differs from those of all other investigators. (See postscript at end of paper.)

Papers bearing on this subject have also lately appeared by Bühler (1902) and Wallace (1903), who describe the changes undergone by newly-discharged follicles in various fishes. Bühler's descriptions, which refer to Cyclostomes and to certain Teleosteans, indicate that there is nothing of the nature of a corpus luteum formed in the cases investigated, while Wallace shows that with the Teleostean Zoarces and the Elasmobranch Spinax there is a very distinct hypertrophy of the follicular epithelium after rupture, thus confirming Giacomini's account (1896) of the recently discharged follicles of certain Elasmobranchs.

Note on the Anatomy of the Internal Genital Organs.

The uterus of the ferret is typically bicornuate, each of the uterine horns passing forward into a slender Fallopian tube, which is very much coiled at its anterior end, passing several times round one side of the ovary. The mouth of the Fallopian tube encloses the ovary, so that the ova on being discharged pass into a sac, and consequently are not shed into the body-cavity. Fig. 8, Pl. 20, represents a transverse section through the ovary, and shows its attachment to the wall of the body-cavity, as well as the sac into which the eggs are shed and the coiled Fallopian tube. The latter appears no less than six times in the section.

The Histology of the Uterus during the Œstrous Cycle.

The changes through which the non-pregnant uterus of the ferret passes during the œstrous cycle may be conveniently arranged according to the same method of grouping as that

employed in describing the similar phenomena occurring in the monkey (Heape, 1894) and the sheep (Marshall, 1903), as follows :

1. Period of rest.
2. Period of growth.
3. Period of degeneration.
4. Period of recuperation.

The changes taking place during each of these periods occur almost simultaneously throughout the whole uterus. Period 1 represents the anœstrum, while the pro-œstrum occurs during Periods 2 and 3. Œstrus, or the period of desire, commences at the close of the period of degeneration, and, as already mentioned, may extend until the end of the recuperation stage, or perhaps even beyond it. Consequently there may be no metœstrum with the ferret, since the period during which copulation can occur is liable to persist until the uterus has reached the resting stage.

1. Period of Rest.—The stroma, of which the greater part of the uterus is formed, is bounded internally by an epithelium consisting of a single row of cubical cells. There is no very clear line of demarcation between the protoplasm of the epithelial cells and the protoplasm of the stroma, neither are there distinct boundaries between the individual cells of the stroma. The latter tissue is fairly uniform in character throughout both the body of the uterus and the two cornua. It contains numerous glands, bounded by epithelia similar to that lining the cavity. Blood-vessels of small size are also present, but are not nearly so abundant as in the succeeding growth stage. Some of these are shown in the figure (Pl. 19, fig. 1), where the general nature of the uterine stroma during the resting stage is indicated.

In comparison with the other stages of the cycle, the uterus at this period may be described as being negatively characterised.

The general shape of the uterine cavity, as it appears in transverse section, is shown in fig. 5 (Pl. 20), which, however, represents a section through an early stage of the growth

period. The same shape and the same general relations between the various layers of tissue are maintained both for the two horns and for the body of the uterus, transverse sections of the latter having a diameter only slightly longer than that of sections cut through one of the horns.

2. Period of Growth.—The beginning of the pro-œstrum is marked by the growth of the uterine stroma, which goes on until the cavity is reduced to about half its normal size. The growth takes place through multiplication of the stroma nuclei, the increase in number occurring for the most part regularly throughout the whole tissue, and not being confined to any particular part. As a result of this process the size of the uterus, as indicated by the length of the diameter of a transverse section through the body or one of the horns, is slightly enlarged, the increased thickness of the walls being not entirely compensated for by the reduction in the size of the cavity.

The multiplication of the stroma nuclei occurs, apparently, by direct division, no mitoses being visible. This appearance is scarcely due to the method of fixation, since evidence of mitotic division can be detected among the cells of the epithelium.

The first indications of growth are followed by an increase in the size of the blood-vessels. At a slightly later stage these also multiply in number, apparently by division of one vessel into two. The increase of the vessels, like that of the nuclei, occurs fairly equally throughout the stroma. The blood-vessels in the surrounding muscular tissue also tend to become enlarged and congested.

Before the close of this period the blood-vessels of the stroma become still further enlarged and packed with corpuscles, while their walls appear stretched, as if preparatory to the breaking-down process which characterises the commencement of the next period.

The epithelium lining the cavity undergoes no material change, though cell-division is perhaps somewhat more frequent. The same may be said of the epithelium of the

glands, which at the beginning of this period undergo a marked swelling, accompanied by greater secretory activity.

3. Period of Degeneration.—Fig. 2 (Pl. 19) represents a portion of a transverse section through the uterus, showing the commencement of the breaking-down process which characterises the period of degeneration. Many of the blood-vessels have their walls still intact, but these are for the most part much congested. Others have apparently just given way, and red corpuscles are already scattered in considerable quantities in the mucosa. Leucocytes are also seen in the tissue outside the vessels, and these probably were extravasated at the same time as the red corpuscles.

The breaking-down process, so far as I have observed, occurs throughout practically the whole of the stoma, and is not confined to the more superficial portion, as in the case of the pro-œstrum of the sheep. The walls of the vessels in the muscular layers, however, do not give way, neither is there any evidence elsewhere of a breaking-down of vessels.

The single layer of lining epithelium during the earlier stages of this period undergoes no change. Subsequently, when nearly all the vessels in the underlying stroma have ruptured, and corpuscles are lying free in most parts of the tissue, indications of degeneration are seen both in the epithelial cells (including those of the glands) and also in the cells of the stroma.

The degeneration of some of the stroma nuclei is accompanied by a tendency on the part of the blood-corpuscles to become aggregated in the more superficial part of the mucosa, where the tissue has become looser, the nuclei being much less densely packed. The process results in the denudation of some portion of the mucosa, and the pouring of little streams of corpuscles into the cavity of the uterus. Meanwhile the glands in the deeper part of the mucosa show an increased secretory activity.

Fig. 6 (Pl. 20) represents a transverse section through one horn of a uterus in which denudation has recently occurred. Most of the blood-corpuscles have already been

got rid of, or at any rate have passed into the lower part of the uterine cavity. Pieces of mucosa, accompanied by corpuscles and mucus, can, however, still be seen lying free in the cavity. A portion of the same section, more highly magnified, is shown in fig. 11 (Pl. 21), where isolated epithelial cells, in a more or less degenerate condition, can be detected among the denuded fragments. In the mucosa forming the uterine wall it is seen that considerable tracts of tissue have been stripped of the lining epithelium, while in some places portions of the underlying stroma also appear to have been removed. Extravasated corpuscles are still seen in the mucosa, but not in any considerable quantity. In some parts of the section there are already indications of recuperation having set in.

I am disposed to believe that there is a not inconsiderable amount of variation in the severity of the pro-œstrous phenomena of the ferret, and that in the case above described the denudation of tissue was exceptional. This was the only example of a ferret killed during the period of degeneration which showed indications of a definite removal of stroma, although a comparison between the thickness of the uterine wall (and, conversely, the size of the uterine cavity) in animals at the beginning of the recuperation stage and during the period of rest also points to the conclusion that destruction is not always confined to the epithelium. In the case of the sheep I found evidence that the severity of the process tended to diminish with each successive diœstrous cycle in the breeding season, so that it is not unlikely that the ferret is subject to some similar variation, depending possibly upon age or upon physical condition.

The chief characteristics of the period of degeneration in the ferret occur in a regular succession almost simultaneously throughout the whole of the uterus, so that this period is capable of subdivision into two or more stages, the first of which is marked by the rupture of the vessels and the extravasation of blood in the stroma. Then further degeneration sets in, and the corpuscles tend to become aggregated

in the proximity of the surface epithelium; and finally, bleeding into the cavity takes place. The whole process, therefore, is very closely comparable to what occurs with monkeys during the degeneration period of menstruation (Heape, 1894, 1897). There is, however, no pro-œstrous clot formed in the ferret's uterus, the discharge seeming to be disposed of very gradually.

4. Period of recuperation.—Fig. 7 (Pl. 20) is a drawing of a part of transverse section under a low magnification, showing the relatively large cavity and correspondingly slight thickness of the mucosa during an early stage of the recuperation period. The epithelium is almost entirely re-formed, but is somewhat attenuated, the individual cells being less columnar in shape than they are normally. Another section through one of the horns of the same uterus is represented in fig. 3 (Pl. 19), which is more highly magnified. This shows that the nuclei of the epithelium are more irregularly arranged than during the other stages of the cycle, while the line of demarcation between epithelium and stroma is even less evident.

The new epithelium is formed, for the most part at any rate, either from that covering certain particular tracts which escaped denudation, or from the epithelium of the glands. I am not quite certain, however, whether the whole of the new epithelium arises in this way, for the absence of a separating line between this layer and the underlying stroma, and the irregular arrangement of the nuclei, upon which I have commented above, suggest that parts of the epithelium may be re-formed from the tissue of the stroma. This is the view adopted by Mr. Heape (1894, 1897) regarding the manner of formation of the new epithelium with monkeys during the recuperative stage of menstruation.

During the earlier stages of recuperation a variable and frequently a large number of red corpuscles, accompanied by wandering cells, remain scattered free in the stroma. These are very numerous in the sections represented in fig. 3 (Pl. 19) and fig. 10 (Pl. 21). At a subsequent stage of re-

cuperation these extravasated corpuscles are no longer seen in any quantity, while numerous small blood-vessels appear to have been formed. In the case of the sheep, it has been shown that the blood which is extravasated during the pro-œstrum, and which is not discharged into the cavity of the uterus, forms pigment in the mucosa. On the other hand, I have never found any trace of pigment formation in the uterine mucosa of the ferret, while sections of this tissue from animals with which recuperation had lately commenced support the view that the corpuscles are gathered up afresh into the circulatory system by becoming enclosed within the walls of newly formed blood-vessels. It is a matter of difficulty in a case of this sort to make quite sure of the correctness of one's interpretation of a series of sections, but unless this explanation, which is in agreement with Mr. Heape's description of what occurs with monkeys, is adopted, I am unable to account for the disappearance of the extravasated corpuscles during the later stages of recuperation.

At a subsequent stage of this period the stroma tissue tends to become more and more dense, and also to increase in thickness, until the mucosa once more acquires its normal condition. This process is effected by the multiplication of the stroma nuclei.

Conclusions.—It is evident, from the foregoing account, that the pro-œstrous process in the ferret is homologous with that of the bitch (Retterer, 1892), the sheep (Marshall, 1902), and the monkey (Heape, 1894, 1897). In severity it is intermediate between the pro-œstrum of the sheep and that of the monkey, while it differs from the same process in the bitch in the somewhat greater denudation of mucosa, at any rate in particular individuals. The "heat" period with the ferret, however, is of considerably longer duration than is the case with the other animals mentioned. Another point of difference from the sheep exists in the absence of pigment formation during the ferret's metœstrum.

The study of the œstrous cycle in the ferret shows very clearly the erroneousness of the view that the degenerative

stage of the pro-œstrum occurs as a consequence of the absence of a fertilised ovum, for which the uterus was preparing, in the preceding growth stage. For, since copulation and ovulation can only take place during œstrus, the uterine denudation occurs prior to the period when fertilisation becomes possible. This is a point to which I have already alluded.

The view that the pro-œstrum is an act of preparation, followed, where this happens to be useless, by a destruction of the preparation, being untenable, I am led to the conclusion that this process is the result of a "wave of disturbance," as Mr. Heape expresses it, which ushers in the period of desire, and is of the nature of a consequence rather than a purpose. On the other hand it appears to me not altogether improbable that the renewal of the mucosa tissue which is consequent upon the degenerative changes may, in some way, help to prepare the uterus for the attachment of the ovum. This view seems to have been entertained by Milnes Marshall (1893).

There is evidence, however, that the pro-œstrous discharge may become not only functionless but even injurious, as in the more severe cases of menstruation in women. This is in accord with the view of Metchnikoff (1903) that the condition of the menstrual flow in the human subject at the present time is essentially a "disharmony" of organisation, and is probably the result of modifications acquired recently in the history of the race. Metchnikoff refers also to the existence of similar disharmonies in the reproductive apparatus of animals, and especially of animals kept in captivity, and probably the severity and long duration of the ferret's "heat" period would be regarded by this author as a further example of the occurrence of such disharmonies.

Summary and Concluding Remarks.

The female ferret is monœstrous, and may have one, two, or three sexual seasons within a year; but although the

œstrous cycle may recur the "heat" periods are usually restricted to the spring and summer months, the autumn and winter being occupied by a prolonged anœstrum. In showing this tendency towards a concentration of sexual seasons the ferret approaches the polyœstrous condition, being in fact, in this respect, intermediate between the dog or cat, which have two, or occasionally three, fairly regularly recurrent œstrous cycles, and the otter, which, in captivity at any rate, has been shown to be polyœstrous with a series of diœstrous cycles, each of a month's duration, occasionally interrupted by a longer anœstrous period.

The pro-œstrum with the ferret may extend for three weeks, while the œstrus, in the absence of the male, may last for another six weeks, or even longer.

The changes which occur in the non-pregnant uterus during the œstrous cycle may be divided according to four periods as follows:

(1) Period of rest.
(2) Period of growth.
(3) Period of degeneration.
(4) Period of recuperation.

The first period corresponds to the anœstrum during which the uterus is in the normal state. This is followed by the growth period during which the uterine cavity becomes reduced to about half its usual size, while the mucosa is correspondingly thickened. Meanwhile the blood-vessels become much congested and subsequently break down, thus marking the commencement of the period of degeneration. The blood-corpuscles become scattered in considerable numbers in the stroma, and eventually in the uterine cavity also, owing to the removal in many places of the lining epithelium. In one specimen I found evidence also of a pro-œstrous denudation of the underlying stroma tissue. Œstrus probably commences towards the close of the period of degeneration, and continues throughout the recuperation stage, or perhaps even beyond it. During the latter period the uterus recovers its normal condition, though the cavity is

at first larger in size than at any other time throughout the cycle.

The character of the changes described affords further proof of the homology between the menstrual cycle of the primates and the œstrous cycle of the lower mammalia, the processes which occur in the uterus of the ferret during the cycle being essentially similar to those which take place in the monkey (Heape, 1894, 1897), the bitch (Retterer, 1892), and the sheep (Marshall, 1903).

Ovulation occurs probably at the commencement of the œstrous period, but only as a result of sexual intercourse. An attempt to induce pregnancy by artificial insemination was a failure, the mere presence of the sperms in the uterus being apparently insufficient to produce the stimulus necessary for ovulation. But while ovulation does not appear to take place in the absence of coition, the œstrus continues for a considerable period after that the time for ovulation has passed by, so that the persistence of the œstrus is associated with the presence of atretic follicles in the ovary.

Since coition and ovulation take place after the pro-œstrum, it is clear that the degeneration stages of the pro-œstrum cannot be of the nature of an undoing, in consequence of the absence of a fertilised ovum, of preparations made during the earlier growth stages.

Fraenkel, however, in a recent paper (1903) adopts the view that the phenomena of menstruation, which has been shown to be homologous with the pro-œstrum, are brought about by the secretory activity of the corpus luteum.[1] This hypothesis, in the light of the facts stated above, appears to

[1] According to Fraenkel the corpus luteum is the organ of internal secretion in the ovary, and controls the nutrition of the uterus, not only during pregnancy, but throughout the whole cycle, there being, properly speaking, but one corpus luteum, which renews itself in slightly different positions, in the case of the human subject at monthly intervals. According to this somewhat extended view of the nature of the corpus luteum, it would seem that the secretions of that organ must be regarded as varying from time to time both in character and quantity, to account for the changes which take place during the uterine cycle.

me to be untenable, while the absence in the ferret's ovaries of corpora lutea (or, at any rate, of newly-formed corpora lutea[1]) during the period of desire, an absence resulting from failure to ovulate, precludes the possibility that œstrus in some way results from an internal secretion of the corpus luteum.

It is important to note in this connection that Mr. Heape found (1897) that not one out of forty-two menstruating females of Semnopithecus entellus had a recently-discharged follicle in either ovary, while one only among seventeen individuals of Macacus rhesus, which were menstruating, had a newly-discharged follicle in one ovary. In this case the monkey was passing through a late stage of menstruation (the stage of the formation of the menstrual clot), while the follicle appears to have been one that had very recently ruptured.

There is, however, a considerable amount of evidence supporting the view that the pro-œstrum is brought about by some kind of ovarian secretion. Thus, it is generally stated that if ovariotomy be performed menstruation ceases, the small percentage of cases where it has been known to continue being accounted for on the supposition that some portion of one of the ovaries was not removed. Moreover, Glass (Halban, 1901) has shown that in the case of a woman with whom menstruation had ceased in consequence of ovariotomy, it was again induced by the grafting of a new ovary. Knauer (Halban, 1901) has performed similar operations on dogs, and similar results were obtained. Halban (1901) also found that after removing the ovaries of monkeys menstruation ceased, while it continued after a grafting of the ovary. Halban's experiments show further that the recurrence of menstruation after the latter operation was not a purely nervous phenomenon, since it took place when the ovary was grafted in a position different from the normal. These and similar observations seem to dispose of the view

[1] In any case, on Fraenkel's hypothesis, the occurrence of the pro-œstrum seems to be entirely dependent upon a previous ovulation.

that the pro-œstrum occurs as a result of ovulation, or is brought about by the pressure of the growing Graafian follicles on the nerve-endings, as supposed by Strassmann (1896).

There are other considerations pointing to the conclusion that the pro-œstrum and œstrus are produced by substances circulating in the blood, though not necessarily secreted by the ovary. Kehrer states that the milk from a suckling sow is affected at the "brunst" period, the young, as a consequence, developing unhealthy symptoms; while similar phenomena have been noted in the case of suckling women during menstruation (Halban, 1901). Youatt (1835) says œstrus can be induced in cows by giving them milk obtained from other cows which are "on heat."

The statements of Ferré and Bestion (Dixon, 1901) that injections of ovarian extract may produce genital excitement have perhaps more direct bearing on this question, but these observations have not so far been confirmed.

Although I am unable, for the reasons stated above, to agree with Fraenkel that menstruation is induced by the secretory activity of the corpus luteum, his experiments, carried on in collaboration with Cohn (1901, 1903), appear to me to go a long way towards establishing the view of these investigators regarding the nature of the connection between the existence of the corpus luteum and the changes taking place in the uterus during gestation. The late Gustav Born had suggested that the corpus luteum was an organ, the function of which was to secrete into the blood substances which prepared the uterus for the attachment and growth of the embryo; and the investigations of Fraenkel and Cohn were undertaken to test this view, to which they lend support. The corpora lutea of rabbits were destroyed by a galvano-caustic needle, when it was found that pregnancy did not continue unless at least one corpus luteum was allowed to remain. Thus the occurrence of pregnancy was shown to depend upon the existence of one or more corpora lutea in the ovary.

It seems possible that the formation of the corpus luteum marks a change in the character of the ovarian secretion, which, in the presence of that structure, may have regard especially to the preparation of the uterus for pregnancy and the attachment of the ovum, and perhaps even the suppression, so to speak, of a pro-œstrous or œstrous secretion during gestation. When, as is sometimes the case with the ferret, ovulation does not take place during the "heat" period, the persistence of the œstrus may possibly be directly correlated with the absence of the corpora lutea.

But whereas such suggestions in the present state of our knowledge are of course highly speculative, the results of recent experiments seem to me to point to the conclusion that the solutions of some of the problems concerning the œstrous cycle and the ripening and final rupture of the Graafian follicles, will be found in the study of the ovary as an organ of internal secretion.

Postscript.

Since concluding the present paper I have read Sandes' account of the formation of the corpus luteum in Dasyurus, of which I had previously only seen an abstract (see p. 330). It is to be noted that this author, although stating in his summary of conclusions that "the corpus luteum atreticum is formed in the same way as the corpus luteum verum," says also that "other atresic follicles are reduced to fibrous tissue or remain cystic." In the body of the paper he describes the former process as occurring only in atretic follicles which had become ripe, or nearly so, but in which the ovum had not been discharged. In the case of the smaller follicles Sandes describes the follicular epithelium as frequently degenerating but never hypertrophying.

Two new articles on the gestation of the badger by Mr. A. H. Cocks have lately been published in the 'Zoologist.' In the last article Mr. Cocks arrives at the remarkable conclusion "that the pairing may take place at any time during

a range of some ten months, and yet that the young are always born within a season limited to about six weeks;" in other words, the gestation period of the badger may be anything between under five and over fifteen months. (See above, page 326, where Meade-Waldo's paper is referred to.)

References to Literature.

Bell.—'A History of British Quadrupeds,' 2nd edition, London, 1874.

Bühler.—"Rückbildung der Eifollikel bei Wirbelthieren," 'Morphol. Jahr.,' vol. xxx, 1902.

Carnegie and other authorities.—'Ferrets and Ferreting,' 3rd edition, London, 1902.

Cocks.—"Note on the Breeding of the Otter," 'Proc. Z. S.,' 1881.

Cocks.—"Habits of the Polecat," 'The Zoologist,' 1891.

Cocks.—"Note on the Gestation of the Pine Marten," 'Proc. Z. S.,' 1900.

Cocks.—"The Gestation of the Badger," 'The Zoologist,' 1903, 1904.

Cohn.—"Zur Histologie und Histogenesis des Corpus luteum und des interstitiellen Ovarialgewebes," 'Arch. f. Mikr. Anat.,' vol. lxii, 1903.

Dixon.—"The Ovary as an Organ of Internal Secretion," 'The Practitioner,' vol. lxvi, 1901.

Fraenkel.—"Die Function des Corpus luteum," 'Arch. f. Gynäk.,' vol. lxviii, 1903.

Fraenkel and Cohn.—"Experimentelle Untersuchungen über den Einfluss des Corpus luteum auf die Insertion des Eies," 'Anat. Anz.,' vol. xx, 1901.

Giacomini.—"Contributo all' istologia dell' ovario dei Selaci," 'Ricerca ve Laboratoria di Anatomia normale della Roy. Universita di Roma, vol. v, 1896.

Halban.—"Ovarium und Menstruation," 'S. B. d. Akad. d. Wissenschaften,' vol. cx, Wien, 1901.

Harting.—"The Polecat, Mustela putorius," 'The Zoologist,' 1891.

Heape.—"The Menstruation of Semnopithecus entellus," 'Phil. Trans. B.,' vol. clxxxv, 1894.

Heape.—"The Menstruation and Ovulation of Macacus rhesus," 'Phil. Trans. B.,' vol. clxxxviii, 1897.

Heape.—"The Artificial Insemination of Mammals, etc.," 'Proc. R. S.,' vol. lxi, 1897.

HEAPE.—"The Sexual Season of Mammals," 'Q. J. M. S.,' vol. xliv, 1900.

MARSHALL, A. MILNES.—'Vertebrate Embryology,' London, 1893.

MARSHALL, F. H. A.—"The Œstrous Cycle and the Formation of the Corpus Luteum in the Sheep," 'Phil. Trans. B.,' vol. cxcvi, 1903.

MEADE-WALDO.—"The Badger: its Period of Gestation," 'The Zoologist,' 1894.

METCHNIKOFF.—'The Nature of Man' (Mitchell's translation), London, 1903.

RETTERER.—"Sur les Modification de la Muqueuse Uterine à l'Epoque du Rut," 'C. R. de la Société de Biologie,' vol. iv, 1892.

ROBINSON.—"Observations upon the Development of the Common Ferret, Mustela ferox," 'Anat. Anz.,' vol. viii, 1893.

SANDES.—"The Corpus Luteum of Dasyurus viverrinus, etc.," Proc. Linnean Society of New South Wales (vol. xxviii, 1903); abstract in 'Nature,' August 20th, 1903.

STRASSMANN.—"Beitrag zur Lehre von der Ovulation, Menstruation, und Conception," 'Arch. f. Gynäk.,' vol. lii, 1896.

WALLACE.—"Observations on Ovarian Ova and Follicles in certain Teleostean and Elasmobranch Fishes," 'Q. J. M. S.,' vol. xlvii, 1903.

YOUATT.—'Cattle,' London, 1835.

EXPLANATION OF PLATES 19—21,

Illustrating Mr. Francis H. A. Marshall's paper on "The Œstrous Cycle in the Common Ferret." The figures were drawn by Mr. J. Taylor, of Edinburgh.

Reference Letters.

b. v. Blood-vessel. *b. v. rup.* Recently ruptured blood-vessel. *cav.* Cavity of uterus (in Fig. 8 cavity of Fallopian tube). *c. t.* Connective tissue of stroma. *ep.* Epithelium. *ep. c.* Isolated epithelial cell. *ep. gl.* Epithelium of gland. *ex. b.* Extravasated blood corpuscles. *gl.* Uterine gland. *leu.* Leucocyte. *musc.* Muscular layers of uterine wall. *ov.* Ovary.

PLATE 19.

FIG. 1.—Transverse section showing portion of uterine mucosa. (Period I.) × ca. 300.

FIG. 2.—Transverse section showing portion of uterine mucosa. (Period III, very early stage.) × ca. 300.

FIG. 3.—Transverse section showing portion of uterine mucosa. (Period IV.) × ca. 300.

FIG. 4.—Transverse section showing portion of uterine mucosa. (Period IV, advanced stage.) × ca. 300.

PLATE 20.

FIG. 5.—Transverse section of horn of uterus. (Period II, early stage.) × ca. 50.

FIG. 6.—Transverse section of horn of uterus. (Period III, advanced stage.) × ca. 50.

FIG. 7.—Transverse section of body of uterus. (Period IV. The entire section is not shown.) × ca. 50.

FIG. 8.—Transverse section of ovary, showing its attachment to the wall of the body cavity, and its enclosure by a sac into which the ova are discharged. × ca. 14. The section passes six times across the coiled Fallopian tube.

PLATE 21.

FIG. 9.—Section through atretic follicle. × ca. 300. The membrana granulosa has almost completely disappeared, while the ovum is much shrunken and in a very degenerate condition. Ingrowth from the connective tissue wall of the follicle has commenced, but has not advanced very far.

FIG. 10.—Transverse section showing portion of uterine mucosa. (Period IV.) × ca. 300. Large numbers of blood corpuscles are seen extravasated in the stroma, while at the same time new (?) blood-vessels are apparently in process of being formed.

FIG. 11.—Transverse section showing portions of uterine mucosa, as well as products of denudation, in the uterine cavity. (Period III, advanced stage.) × ca. 300.

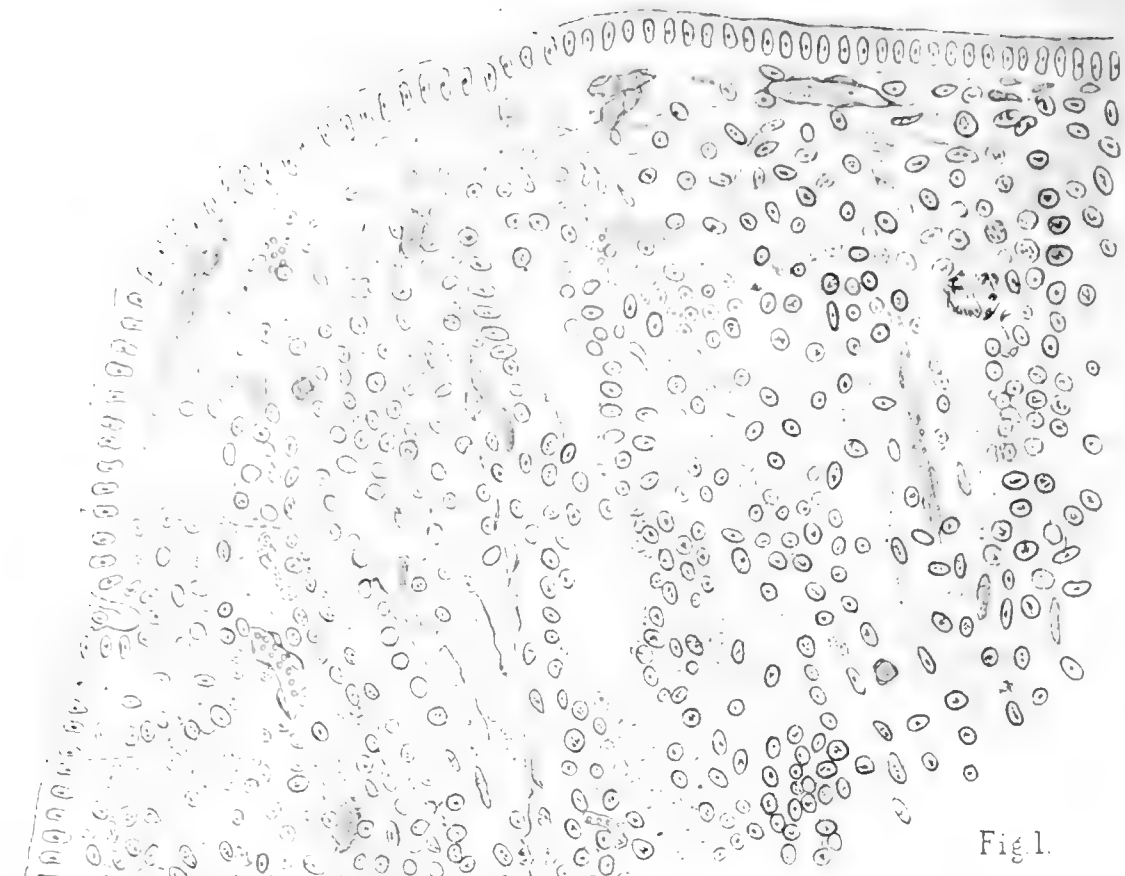


Fig. 1.

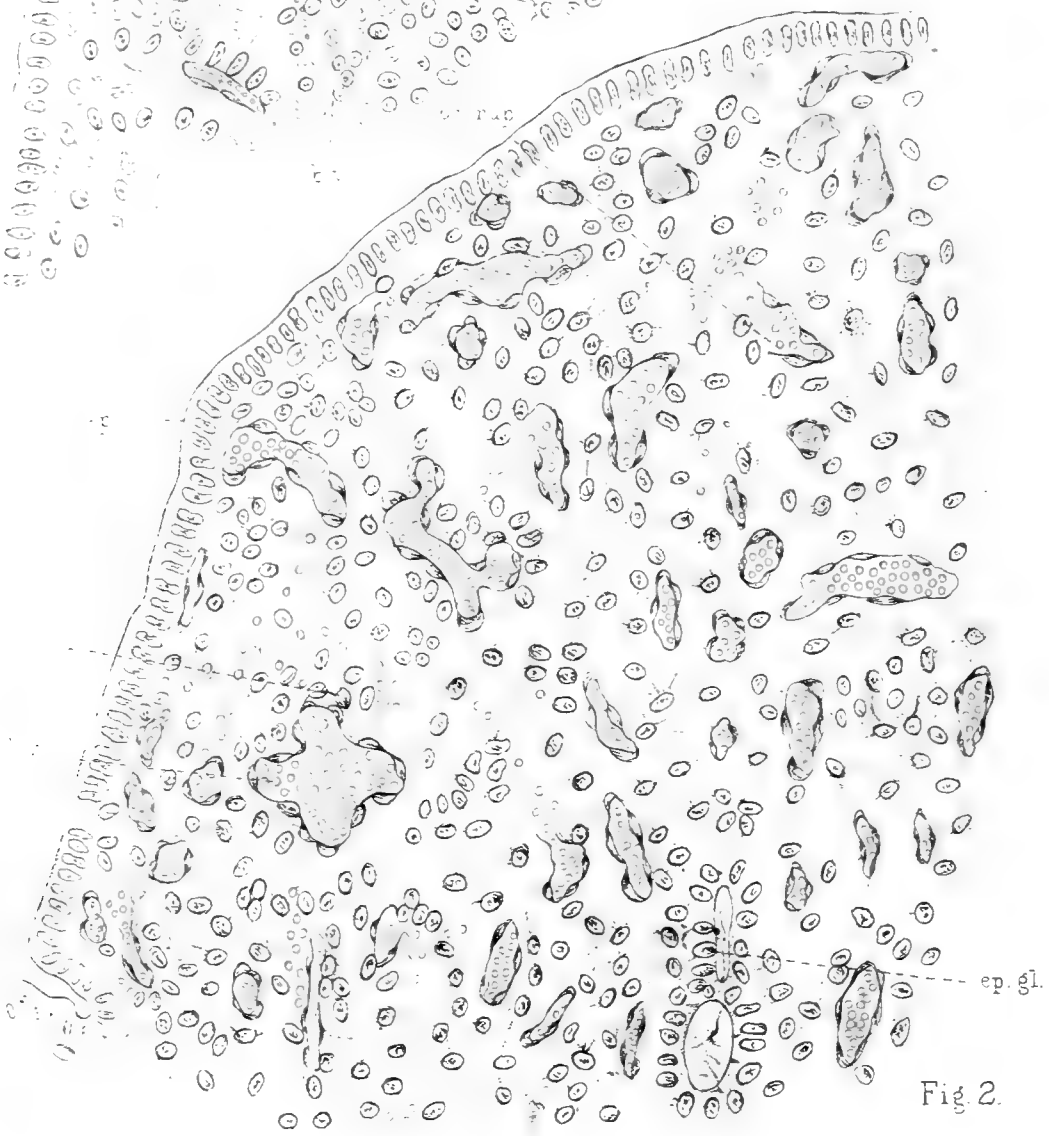


Fig. 2.

Fig 3.

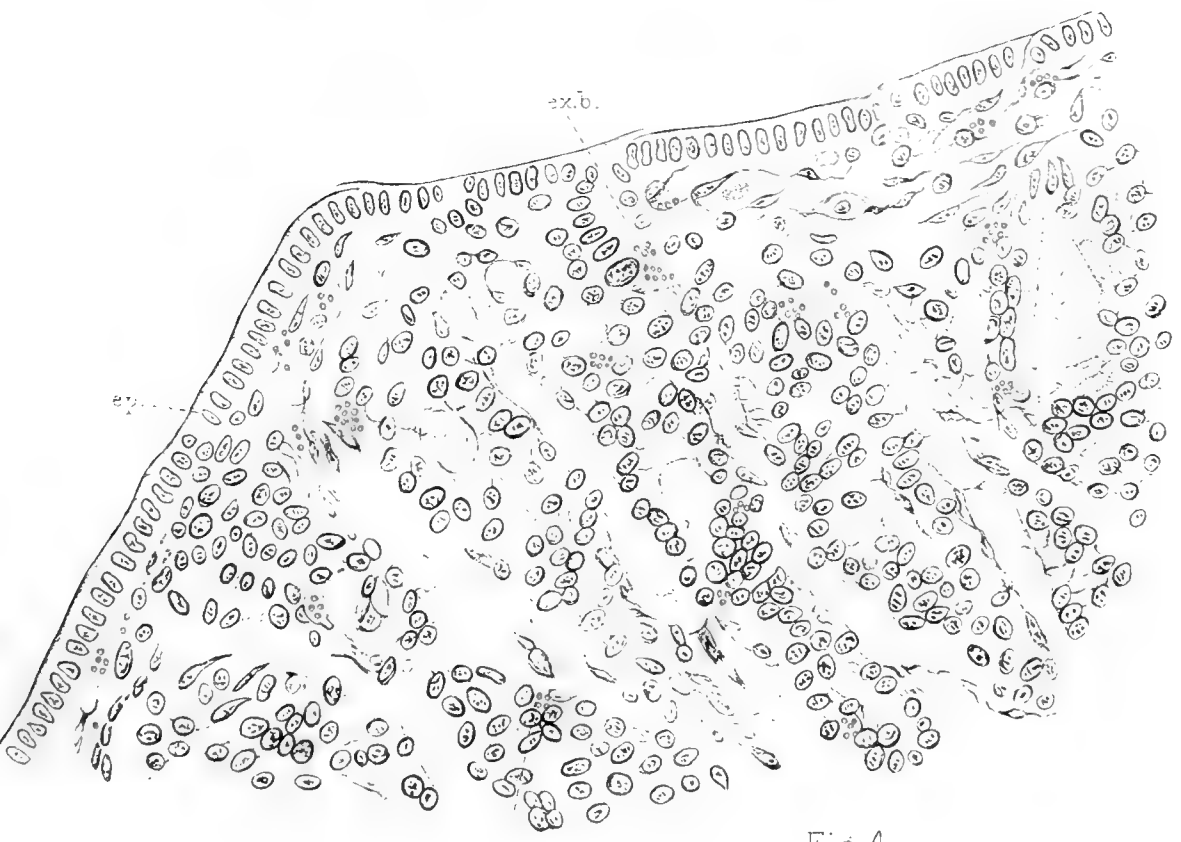
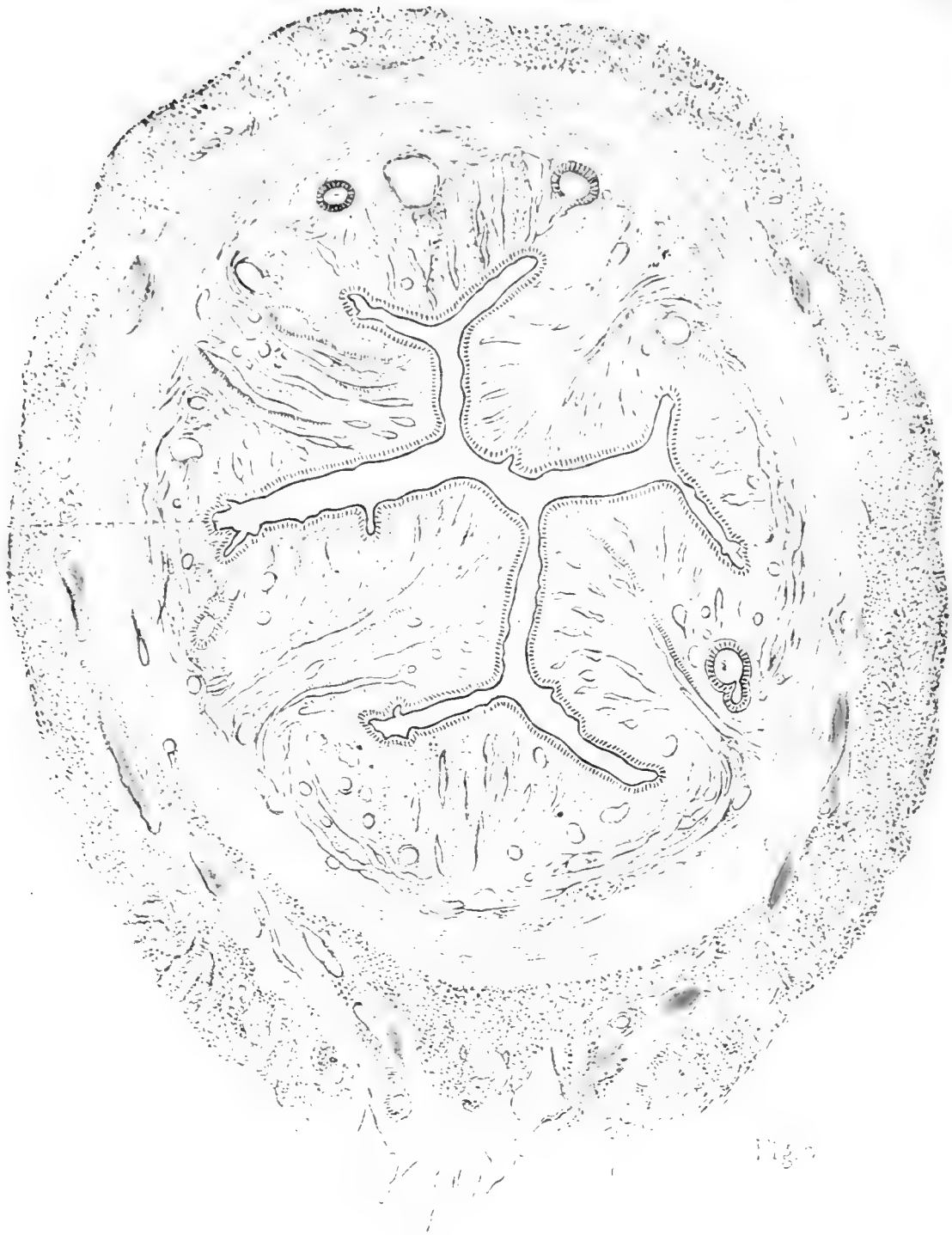


Fig 4.



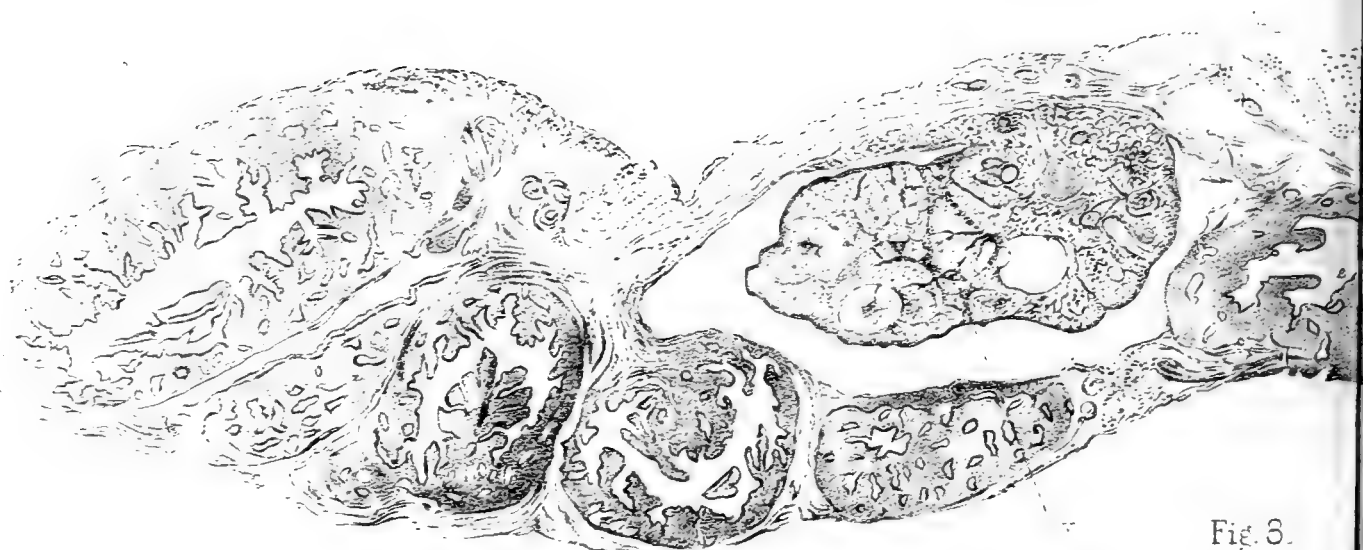


Fig. 3.

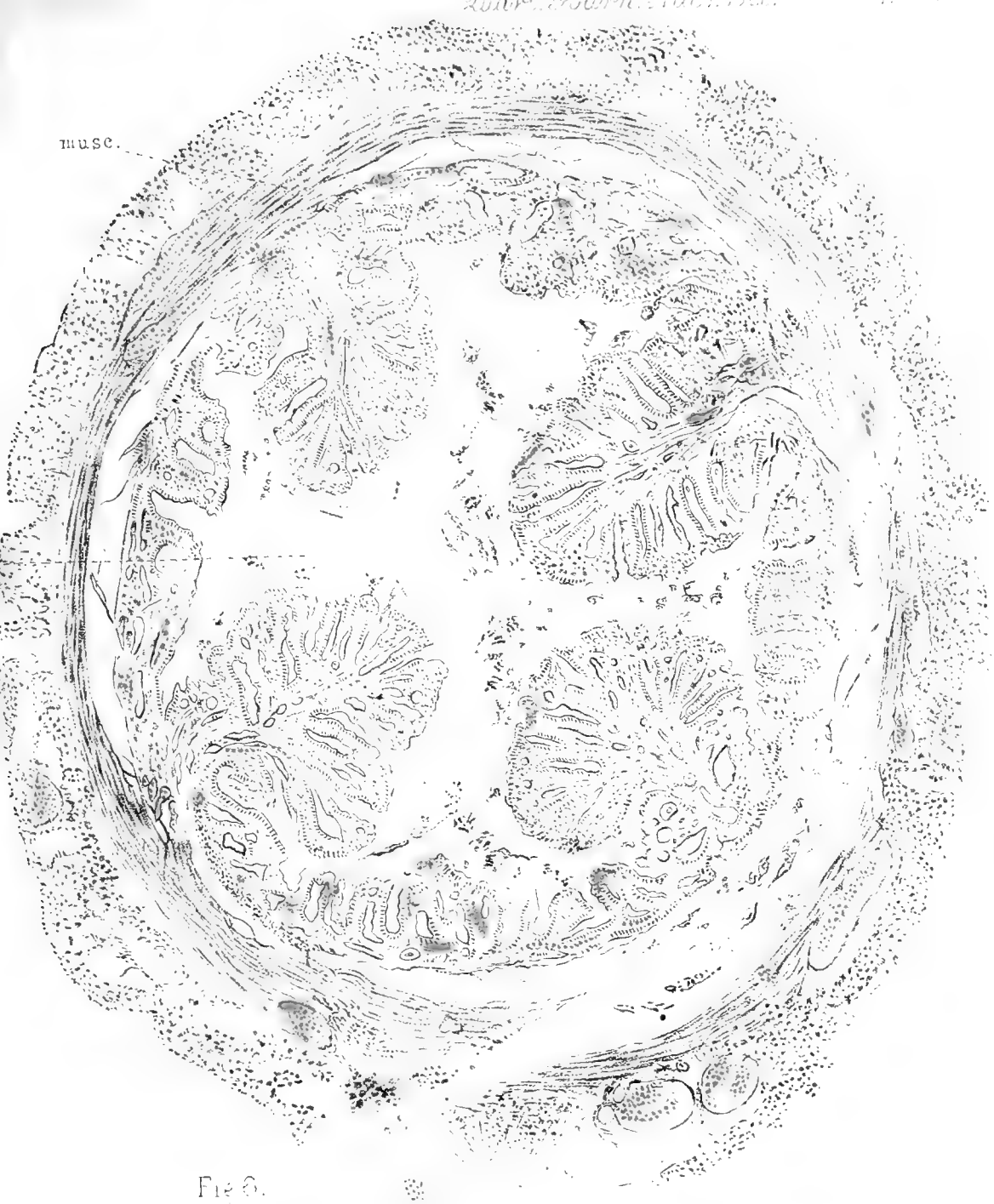


Fig 6.

Fig 7

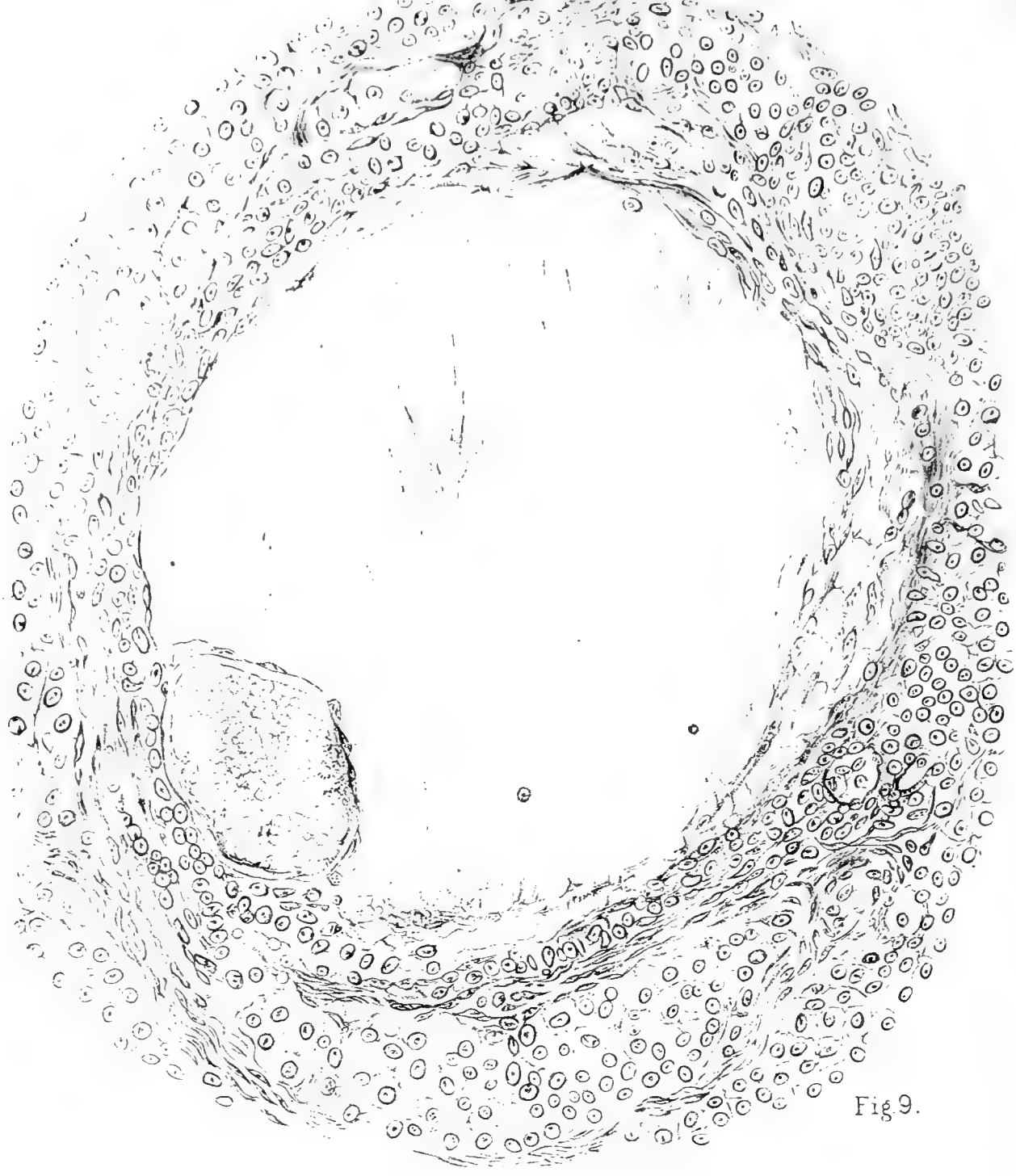


Fig.9.

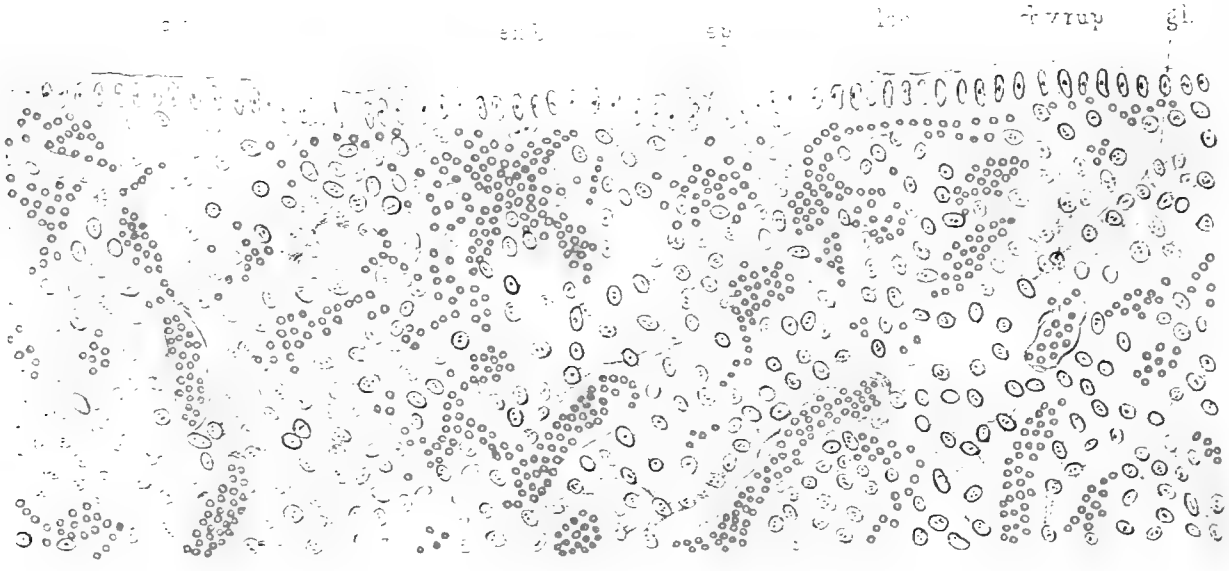


Fig.10.

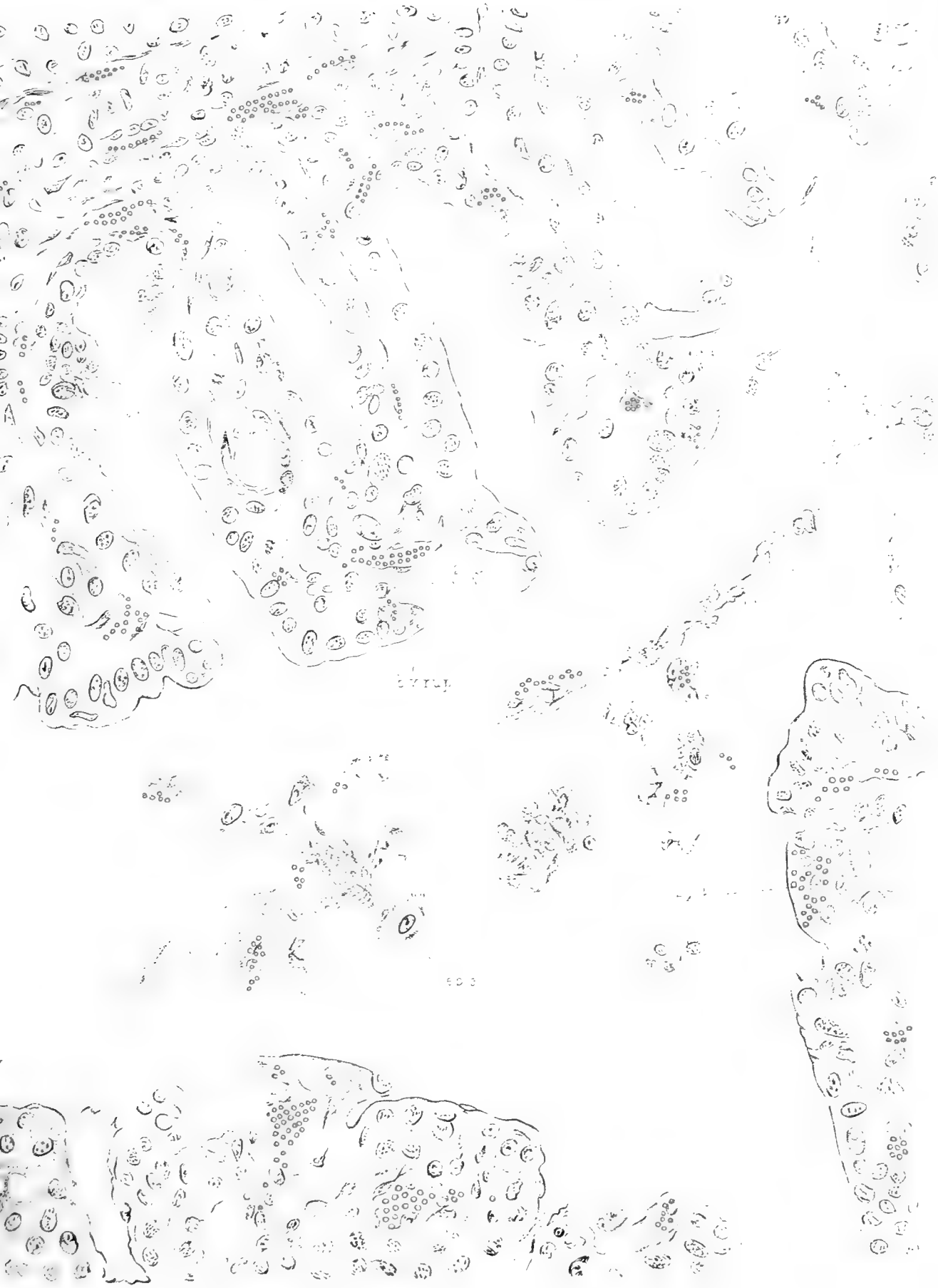


Fig II.

Heath, Litht. London.

Two New Forms of Choniostomatidæ: Copepoda Parasitic on Crustacea Malacostraca and Ostrocoda.

By

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(Copenhagen).

With Plate 22.

During a stay—in August, 1902—in the house of my friend, the Rev. Canon A. M. Norman, I had the good luck to discover, in his splendid collection, a new parasitic Copepod on a couple of specimens of a rare Norwegian Amphipod. He asked me to work out the parasite, which I promised, and the result is given here.

Since the present writer, six years ago, published the book, 'The Choniostomatidæ,' Copenhagen, 1897 (4to), no species has been added to this rich and very interesting family of parasitic Copepoda, though the animals do not seem to be so rare as probably generally believed. In the preface to the book named I stated that in the course of 1895–97 I had found, "on the material brought home from the sea near Iceland and Greenland by the 'Ingolf' expedition, several new forms which cannot be included in the present treatise," and since 1897 I have accumulated additional material. During the study of the species from Canon Norman's collection I felt that a paper dealing only with that single form

would be rather meagre, but for various reasons it was impossible for me to work out the whole new material collected since 1895. Under these circumstances I resolved to embody here one very interesting form, secured on animals from New Zealand, and extending in various respects our knowledge of the family. "The Choniostomatidæ, a family of Copepoda, parasitic on Crustacea Malacostraca" is the complete title of my above-mentioned book, but the new form from New Zealand of the same family lives on a species of Ostracoda, an order of Crustacea Entomostraca. Besides, nobody has ever, as far as I know, found any Copepod parasitic on any species of Ostracoda, while on three forms belonging to this order G. O. Sars has discovered one, and Th. R. R. Stebbing a second, and G. W. Müller a third species of the Cryptoniscinæ (a sub-family of parasitic Isopoda); the two first-named authors have produced full description of the parasites, while that found by Müller was left undescribed.

The following descriptions of the two forms were written nearly in conformity to those in my earlier monograph.

Sphæronella norvegica, n. sp.

Pl. 22, figs. 1*a*—1*g*.

Female.—The specimen drawn (fig. 1 *a*) and dissected measured 1·7 mm. in length, and nearly the same in breadth; the two other specimens seen are almost similar in size. The head is proportionately very small and well-defined from the trunk.

The frontal margin (fig. 1 *d*) is well developed, adorned with a row of very short hairs, but the most lateral portion of the margin, towards the base of the antennulæ, is naked. The antennulæ (*a*) are long, three-jointed, with the terminal setæ very long; the third joint has, a little before the middle, an olfactory seta (*s*), which is a little shorter than the terminal ones. The antennæ (*b*) are of moderate length, three-jointed; the joints decreasing strongly in length from the first to the

third, which terminates in a seta. (In the specimen dissected the left antennæ had been lost, while the right one is shown on the left side of the figure.) The mouth is good-sized, with a broad border. The maxillulæ (*d*) with a well-developed "additional branch" (d^1). The maxillæ (*e*) are large; the basal joint is robust and long, with a rounded protuberance on the lower side, a little from the base and from the outer side, but without hairs, excepting the usual row along the distal margin. The maxillipeds (*f*) are long; the basal joint moderately robust without hairs; the three other joints are well marked off from each other; the terminal one is slender, and terminates in a few exceedingly fine, spiniform processes. The submedian skeleton presents two pairs of conspicuous chitinous strips; each of these is sinuate, and, besides, anteriorly bent very sharply in an acute angle, thus forming an outer short strip turning outwards and backwards; the subtriangular space between the outer set of these strips, situated near the posterior admedian angle of the maxillæ, is adorned with very long hairs, and some moderately long hairs are also found at the posterior margin of the maxillæ. Behind the maxillipeds two transverse strips are seen; rather near the middle the strips on the same half of the animal run together, forming a single strip which bends backwards and inwards, uniting itself in the middle line with the corresponding one from the other half; the front one of these transverse strips is adorned with some long hairs, and close to the posterior strip or a little removed from it some similar hairs are seen. The lateral margin of the head with a fringe of moderately long hairs. On the dorsal surface, a little behind the frontal margin, a rather small, oblong, transverse area, set with numerous very short hairs, is seen about equidistant from the middle line, and from the base of the antennula. The trunk is on the anterior half, and especially on the anterior third, set with a good number of rather conspicuous, simple, stiff hairs; on the posterior half these hairs are much less numerous, shorter, and not easily observed; the trunk-legs are exceedingly small, but one of each pair was found.

The genital area (fig. 1 *c*) is a little broader than the head (it is not visible in fig. 1 *a*, being situated above and in front of the posterior outline of the body); it is a transverse plate with six rounded angles, but not very regularly shaped; the posterior margin of the plate is straight, about as long as the postero-lateral margin, which is longer than the antero-lateral one; the front margin is wanting, the large anterior middle portion of the area being occupied by membranous skin. The plate is adorned with a moderate number of short, stiff hairs, each originating from a conspicuous "foot," and these hairs are spread very irregularly. The genital apertures (*g*) are rather curved, and they diverge a little with their anterior third; they are placed in the penultimate fourth part of the plate, and the distance between them at the middle is somewhat shorter than the length of one of them. The caudal stylets (*st*) are situated close together, at a good distance in front of the posterior margin of the plate, a little behind the genital apertures.

Male.—The single specimen (figs. 1 *b*, 1 *f*, and 1 *g*) measures ·3 mm. in length, and ·213 mm. in breadth; it is thus nearly six times shorter, and between seven and eight times narrower than the female. Seen from below the head occupies a little more than one third of the length; its lateral outlines from the antennæ backwards diverge rather feebly, but where the subglobular trunk begins the lateral margins begin to diverge strongly. The frontal border has a fringe of fine hairs. The antennulæ are rather slender, of moderate length, very distinctly three-jointed; the setæ on the distal front angle of the first joint, and especially those at the apex of the third joint are long. The antennæ (*b*) are rather well developed, nearly as in the female; the basal joint is much longer than the second, which is longer than the third, the latter one is short and terminates in a rather short seta. The border of the mouth is moderately broad. The maxillulæ (*d*) have a well-developed additional branch (d^1). The maxillæ (*e*) are medium sized, the basal joint with a rounded protuberance on the posterior side. The submedian skeleton has a chitinous

longitudinal strip at the inner base of the maxillæ, and this strip is posteriorly scarcely produced into a free process; from the anterior part of the inner margin of this strip projects another less developed shorter strip backwards and a little inwards; no processes are found between the maxillipeds, but behind their insertions is seen a narrow, transverse strip, which, at the middle, is curved a little backwards, and behind this strip still another very narrow strip, interrupted at the middle, constitutes the limit between head and thorax. Inside the postero-interior angle of each maxilla a short transverse row of long hairs is found. The maxillipeds (*f*) consist of four distinct joints; the basal joint is rather long and slender without hairs; the fourth joint has the end obtuse, and adorned with a few fine spines. The lateral margin of the head has a row of moderately long hairs, and this row begins above the insertion of the antennula; the head has besides a short oblique, transverse, dorsal area with rather short hairs inside and a little in front of the anterior end of the insertion of each antennula (fig. 1 *g*), and a short row of hairs on the dorsal surface rather near the middle line and somewhat in advance of the thorax. The trunk is everywhere, with exception of a narrow and badly-defined transverse belt at the base on the lower surface, clothed with rather long setiform hairs, which show an interesting structure. From tiny transverse chitinous knots two or three hairs originate, and the middle one is much longer than the others; besides, the knots are arranged in short or moderately long, more or less regular, transverse, or somewhat oblique rows; the length of this clothing is about the same on all parts of the trunk and nearly as long as the diameter of the basal joint of the maxillipeds. The two pairs of trunk-legs are nearly similarly shaped, both consisting of a single truncate joint; the joint of the first pair (l^1) is somewhat longer than thick, and not as thick as that of the second pair (l^2), which is as long as thick; the joint of the first pair terminates in two setæ, one only a little shorter than the basal joint of the maxillipeds, while the other seta is about three times shorter; the joint of

the second pair with two nearly similar setæ, but its long seta is scarcely as long as that on the first pair of legs. The caudal stylets (*st*) are rather similar to the second pair of legs, each terminating in two or three setæ, the longest of which is a little shorter than the corresponding one of the legs, while the other setæ are more than half as long as the long seta.

Ovisacs.—The ovisacs belonging to two females differed little in size, while those of a third female differed considerably from each other, but that was to a certain degree due to the different stage of development of their contents. The two ovisacs figured (fig. 1 *c*) give the average size of them as compared with the female (fig. 1 *a*) and the male (fig. 1 *b*), all being drawn with the same degree of enlargement; the large one of these ovisacs measures nearly ·7 mm. in diameter. The ovisacs are generally subglobular, sometimes irregularly flattened from pressure; each contains a rather high number of eggs.

Larva and Post-larval Development.—Unknown.

Habitat.—Among several specimens of Rhachotropis leucophthalmus, G. O. Sars, secured by Canon A. M. Norman in Throndhjemsfjord (Norway) from a depth of 250—300 fathoms, I found two adult females with this parasite. In one marsupium I found one female, one male and twelve ovisacs, the latter ones all adhering to each other; in the other marsupium was one female with six free ovisacs. The single male was very dirty, and, hoping to get some fine specimens, I applied myself to Prof. G. O. Sars, who, with his usual courtesy, lent me his whole material of that rare Amphipod for inspection. I found only one infested specimen, with one female, eight free ovisacs, but no male. (I succeeded afterwards in cleaning the male rather well with two brushes, each consisting of one short and fine hair fixed in a small stick).

Remarks.—The male is large in proportion to the same sex in most other species of Sphæronella, and it is much larger than one of the eggs; by the shape of the legs and

the length of the seta on these appendages and on the caudal stylets it differs considerably from all other forms hitherto known. Furthermore, I have not observed the existence of two hairy areæ in front and two transverse rows of hairs more backwards on the upper surface of the head in the male of any other species. The structure of the hairs on the trunk is rather similar to that met with in Sph. Giardii, H. J. H. The female is, as usual, less characteristic than the male, but presents yet some distinguishing features: in most other species the trunk is almost totally naked; in no other female I observed two hairy areas on the upper surface of the head behind the frontal margin, and the distribution of hairs on the lower surface of the head is rather similar to that in Sph. intermedia, H. J. H., but differing from most other species; the shape of the submedian skeleton and of the transverse strips just in front of the trunk was not observed in any other form.

Sphæronellopsis, n. gen.

Female.—The body is more or less ovate. The head is rather large, well defined from the trunk. The frontal border is at most feebly developed, while the lateral margins are wanting. The antennulæ are small, two-jointed; the antennæ wanting. The mouth of moderate size; its border is narrow. The maxillulæ are well developed, with a good-sized additional branch. The maxillæ are rather small, but normally shaped. The maxillipeds consist of only three joints: the basal one is short, but very thick, inflated; the second joint, which certainly is formed by the complete fusion of two joints, is rather short; the terminal joint is nearly rudimentary. The trunk has two quite rudimentary pairs of legs, each consisting of a joint with one terminal seta. The genital area is well developed, situated on the posterior surface of the body, nearly as long as broad; the genital apertures are situated as in Sphæronella rather

near each other and in the posterior part of the area, but from its anterior (lower) portion a broad, low protuberance (fig. 2 *g*, *p*) is directed downwards, the lower rounded or rather truncate end of which protrudes freely and conceals the anterior (lower) margin of the area itself and a small portion of the skin in advance of that area. The receptacula seminis (fig. 2 *g*, *r*) are very long, slender, sausage-shaped, and very curved, situated beneath the middle of the area, and their entrances, which could not be distinguished with certainty, must be rather near the genital apertures. The caudal stylets (fig. 2 *g*, *st*) are completely fused with each other in nearly their whole length.

Male.—Unknown.

Ovisacs.—As in Sphæronella, and deposed freely.

Larva and Post-larval Development.—Unknown.

Habitat.—The upper posterior space between the shells of Ostracoda, hitherto found only at New Zealand.

Remarks.—Unfortunately only the female and the ovisacs of one species are known, while the male is unknown. The female is similar and closely allied to the rich genus Sphæronella, but differs in the following features: the fusion of the caudal stylets, the genital area being furnished with a large protuberance, and the sausage-shaped, strongly curved receptacula seminis. Besides, the habitation of the parasite on Ostracoda is a most remarkable feature.

Sphæronellopsis littoralis, n. sp.

Pl. 22, figs. 2*a*—2*g*.

Female.—The largest specimen (fig. 2 *b*), which scarcely had begun to deposit ovisacs, measured ·57 mm. in length to the end of the projecting mouth and ·41 mm. in breadth, but the body was rather depressed; another similarly depressed female (fig. 2 *a*), found together with eight ovisacs and with a much smaller half evacuated female, measured ·48 in length

and ·4 mm. in breadth, but in this specimen the mouth turns essentially downwards. The head is sharply defined from the thorax, broader than long, without lateral borders. The antennulæ (fig. 2 *e*) are small, two-jointed, the second joint not well defined, as long as or shorter than the first, with an olfactory seta (*s*) nearly longer than the whole antennula, and besides with two, three, or four acute, somewhat shorter setæ. The maxillæ (*e*) have no protuberance or hairs on the rather slender basal joint; the second joint is thick at the base. The maxillipeds (*f*) are rather anomalous; the basal joint is short, but exceedingly thick, only very little longer than thick, with the admedian margin concave and the outer side strongly vaulted; a transverse row of short hairs is seen on the inner part of the lower side on its proximal half, and a similar and little longer row at the distal margin near the articulating membrane. The second joint is rather short, and not completely regularly shaped; the third joint is shaped nearly as a short thick claw inserted on the anterior surface of the second joint near its end. The submedian skeleton is not very strongly developed, without processes. Head and thorax without hairs. The genital area (fig. 2 *g*) is about half as broad as the base of the head, nearly as long as broad, with the outline almost circular, but somewhat emarginate behind; the protuberance (*p*) mentioned in the diagnosis of the genus is well chitinised, especially the lateral parts of its proximal half, but the lateral part of the area itself is less chitinised, and the portion in the main covering the muscle of each half, is rather thin-skinned. The protuberance is distally either rounded or truncate, with the angles rounded. The genital apertures (*g*) are long, strongly curved, their most anterior (lower) portion is nearly parallel, and the distance between them is here slightly more than one half of their length, while the distance between their opposite ends is about two and a half times longer than one of them. The whole area is naked. The muscles (*m*) to the antero-lateral half of the frame of each aperture are directed somewhat outwards and essentially forwards. The sausage-

shaped, strongly sinuate receptacula (*r*) are situated at the admedian margin of the muscles; on the figure the two receptacula are very differently curved; they were drawn in the position observed, but I believe that the receptaculum on the left half of the figure is the normal one. The caudal stylets (*st*) are fused with each other, together a little broader than long, more or less incise behind, and from the end of each half originates a comparatively strong seta which is from two to almost three times longer than the stylets; these are inserted on the posterior (upper) margin itself of the chitinised area.

Male.—Unknown.

Ovisacs.—They differ very considerably in size, and are subglobular, shortly ovate or somewhat flattened. I have figured, with the same degree of enlargement, one of the smallest (fig. 2 *c*) and one of the largest (fig. 2 *d*) ovisacs together with the largest of the two females found together with them; the greatest dimension of the smallest ovisac is ·2 mm., of the largest one ·27 mm. The eggs are proportionately large, in one of the smallest ovisacs about eight or nine, in a large one between twenty and thirty.

Larva and Post-larval Development.—Unknown.

Habitat.—Years ago I discovered this species in three specimens of the Ostrocod Sarsiella hispida, Brady, from Akaroa Harbour (New Zealand), six fathoms. In two specimens I found only a female without ovisacs, in the third specimen one rather large female, one very small, half evacuated female, and eight ovisacs. Between several hundreds of Sarsiella Hanseni, Brady, from Lyttleton Harbour (New Zealand), one to five fathoms, I found a good number of Sarsiella hispida, Brady, and two of these infested, in one specimen a female without ovisacs, in the other one very small female with nine ovisacs. The parasite, surrounded by its ovisacs, is placed in the posterior upper half of the space between the shells, essentially above the posterior half of the body of the Ostracod; some of the ovisacs were also found within the hollow pro-

tuberances projecting from the upper postero-lateral angles of the shell in that species of Sarsiella. I looked in vain for males. It is interesting that while several infested specimens of S. hispida were discovered, I did not find the parasite on any specimen of S. Hanseni, which was taken together with the other form, but was much more abundant, and I have inspected a good number of the latter species. Prof. G. S. Brady established both species of Ostracoda on material from the Copenhagen Museum.

Remarks.—The rich material of both species of Sarsiella was procured to our Museum in the following way. I wrote an instruction to H. Suter, Esq., how he should deal with the bottom material and send it to us preserved in spirit; in sieved mud received from him I found a good number of tolerably preserved specimens of these Ostracoda and of numerous other Crustacea, many of which were new to science. The above-described parasite must be rather easy to secure by zoologists living in New Zealand or staying there during some time. I will exhort these colleagues to take up the investigation and look for males and stages of development. I suppose that if my own material had been somewhat better preserved or still richer I should have been able to find these tiny animals, which probably were fallen out before my inspection. My earlier monograph of the family gives full information on the mode of proceeding applied by me in order to find and deal with such minute forms without damaging them. I am inclined to believe that several species of Ostracoda inhabiting other places in the world are infested with hitherto unknown species of Sphæronelloides. Our knowledge of parasitic Copepoda is still in its infancy, and numerous interesting, even startling, discoveries in this field are to be done by zoologists in the future.

EXPLANATION OF PLATE 22,

Illustrating Mr. H. J. Hansen's paper on "Two New Forms of Choniostomatidæ."

FIG. 1.—Sphæronella norvegica, n. sp.

FIG. 1 *a*.—Female, from below. × 28. l^1, leg of the first pair; l^2, leg of the second pair.

FIG. 1 *b*.—Male, from below. × 28.

FIG. 1 *c*.—Two ovisacs. × 28.

FIG. 1 *d*.—Head of the female, from below. × 322. *a*. Antennula. *b*. Antenna. *c*. Mandible. *d*. Maxillula. d^1. Additional branch of the maxillula. *e*. Maxilla. *f*. Maxilliped. *s*. Olfactory seta on the last joint of the antennula.

FIG. 1 *e*.—Genital area of the female. × 182. *g*. Genital aperture. *m*. Muscle to the outer strip of the frame around the aperture. *r*. Receptaculum seminis indicated with dotted outline, as seen through the skin (the other receptaculum is omitted). *st*. Caudal stylet.

FIG. 1 *f*.—Male, from below. × 170. *b*. Antenna. *d*. Maxillula. d^1. Additional branch of the maxillula. *e*. Maxilla. *f*. Maxilliped. l^1. Leg of first pair. l^2. Leg of second pair. *st*. Caudal stylet.

FIG. 1 *g*.—Head of the same male, from the side. × 222. The lettering as in Fig. 1 *f*.

FIG. 2.—Sphæronellopsis littoralis, n. gen., n. sp.

FIG. 2 *a*.—Female, from below. × 71. *g*. Genital area.

FIG. 2 *b*.—Large female, with the head directed forward. × 71. Of the trunk-legs, only those on the right side of the figure are shown.

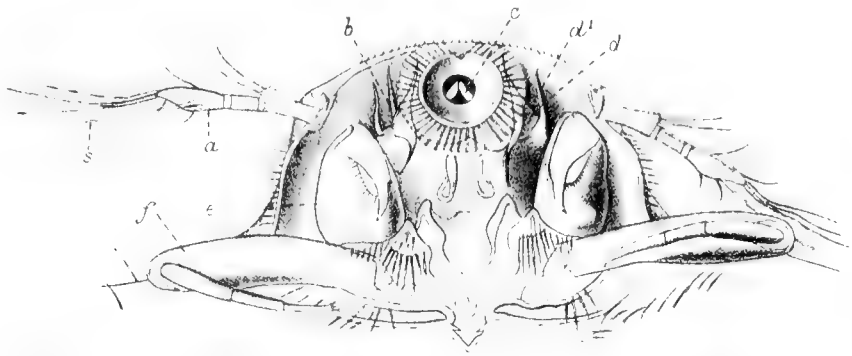
FIG. 2 *c*.—Small ovisac. × 71.

FIG. 2 *d*.—Large ovisac. × 71.

FIG. 2 *e*.—Head of a female, from below. × 240. The lettering has the same significance as on Fig. 1 *d*.

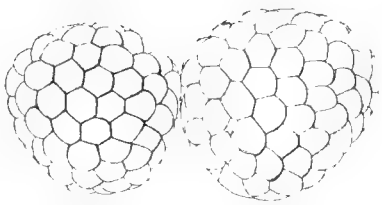
FIG. 2 *f*.—Anterior part of the head of the female represented in Fig. 2 *b*, showing the border of the mouth and the maxillulæ, with their three branches or setiform processes. × 285.

FIG. 2 *g*.—Genital area of a female. × 325. *g*. Genital aperture. *m*. Muscle opening that aperture. *p*. Anterior (lower) end of the large protuberance, met with only in this genus. *r*. Receptaculum seminis. *st*. Caudal stylets fused with each other.

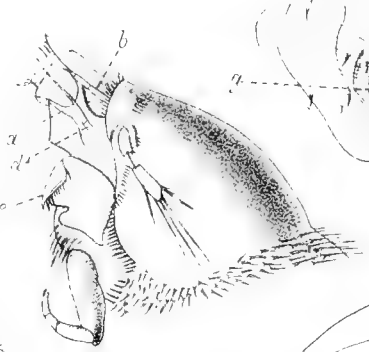


1d.

1b.

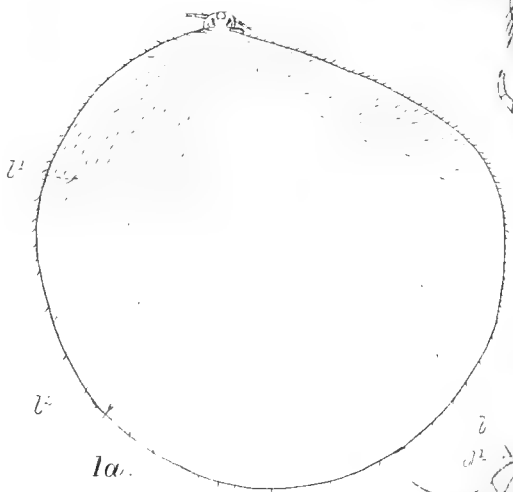


1c.



1g.

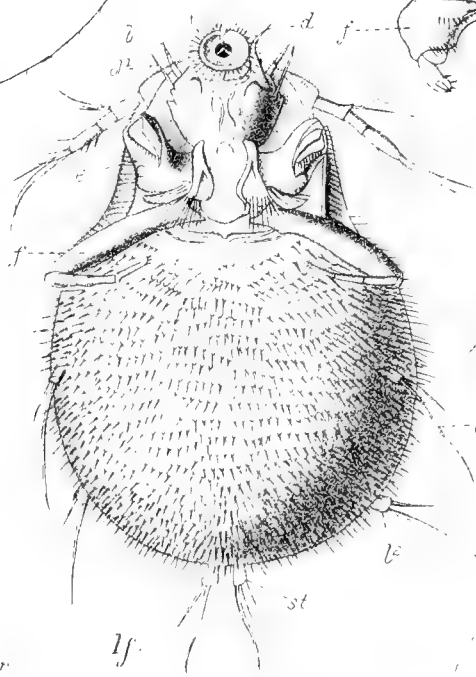
1e.



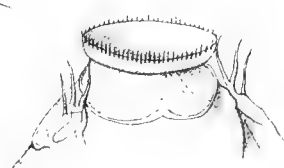
1a.

2e.

2a.



1f.



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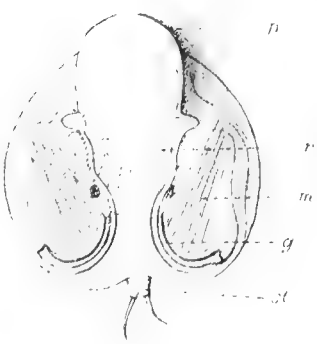
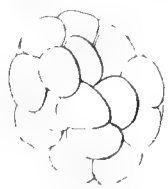
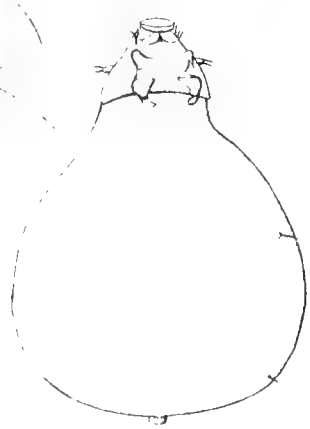


2g.

2c.



2d.



2b.

H. J. Hansen del.

Huth. Lith.ᵗʰ London.

1. Sphæronella norvegica n. sp. 2. Sphæronellopsis littoralis n. gen., n. sp.

On the Existence of an Anterior Rudimentary Gill in Astacus fluviatilis, Fabr.

By

Margery Moseley.

With Plates 23 and 24.

The theoretical gill formula for the Decapod crustacea is four on each side of each somite, corresponding to the three maxillipeds and the five legs—that is, Somites VII to XIV, counting the ophthalmic somite as No. 1.

Professor Huxley distinguished the four gill plumes according to their position on the somite. He recognised one podobranch on the limb, two arthrobranchs on the arthrodial membrane, and a pleurobranch on the pleuron or side of the somite between the leg-joint and the tergum.

The complete theoretical gill formula according to Huxley for one side of the animal would be—

Somite.	Podo-branchiæ.	Arthro-branchiæ.	Pleuro-branchiæ.	Total.
VII	1	2	1	4
VIII	1	2	1	4
IX	1	2	1	4
X	1	2	1	4
XI	1	2	1	4
XII	1	2	1	4
XIII	1	2	1	4
XIV	1	2	1	4
				32

The nearest approach to this is found in certain Penæidæ,

belonging to the subfamily Aristæinæ. Alcock[1] gives the following formula for the subgenera Plesiopeneus, Aristæomorpha and Aristæopsis of the genus Aristæus and for the genera Benthesicymus and Gennadas.

Somite.	Podobranchiæ.	Arthrobranchiæ.	Pleurobranchiæ.	Total.
VII	0 (ep.)	1	0	1+ep.
VIII	1 (ep.)	1	1	3+ep.
IX	1 (ep.)	2	1	4+ep.
X	1 (ep.)	2	1	4+ep.
XI	1 (ep.)	2	1	4+ep.
XII	1 (ep.)	2	1	4+ep.
XIII	0 (ep.)	2	1	3+ep.
XIV	0	0	1	1
				24+7 ep.

A practically identical formula is given by Boas[2] for the aberrant Penæid Cerataspis longiremis.

The formula given by Professor Huxley for Astacus fluviatilis is as follows[3]:—

Somite.	Podobranchiæ.	Arthrobranchiæ.	Pleurobranchiæ.	Total.
VII	0 (ep.)	0	0	ep.
VIII	1	1	0	2
IX	1	2	0	3
X	1	2	0	3
XI	1	2	0 or r	3+0 or r
XII	1	2	r	3+r
XIII	1	2	r	3+r
XIV	0	0	1	1
				18+ep+2r or 3r

[1] 'Descr. Catalogue Indian Deep-Sea Crustacea,' pp. 35, etc. (1901).

[2] 'Vidensk. Selsk. Skr. 6 Raekke, naturvid. math. Afd.,' i (2), p. 43 (1880). *Cf.* also Claus, 'Arb. Zool. Inst. Wien,' vi, p. 49 (1885) and Bounier, 'Trav. Stat. Zool. Wimereux,' vii, p. 38 (1899).

[3] Huxley does not enumerate the epipodites accompanying the podobranchs as is done in the formula quoted above from Alcock.

He recognised two kinds, the stone-crayfish and the noble-crayfish, which he called Astacus torrentium (Schrank), and Astacus nobilis (Huxley). He mentions that A. torrentium never has more than two rudimentary pleurobranchs, whereas he had found three in A. nobilis. The stone-crayfish A. torrentium was the same as that found in England, and he left it an open question whether they were both varieties of A. fluviatilis, or whether they were specifically different, in which case A. nobilis was the true A. fluviatilis.

Of A. leptodactylus, and the closely allied forms A. pachypus and A. angulosus, Professor Huxley says that "if A. angulosus and A. pachypus are varieties of A. leptodactylus, I cannot see why Gerstfeldt's conclusion that A. nobilis is another variety of the same form need be questioned on morphological grounds." Faxon[1] and Ortmann[2] recognise the following European species: Astacus fluviatilis, Fabr.,[3] A. leptodactylus Esch., A. pallipes Lereb.,[4] A. torrentium Schrk., A. pachypus Rthke. and A. colchicus Kessl., which differ from each other not only in colour and in the form of the rostrum and limbs, but also in some cases in the number of rudimentary pleurobranchiæ in the hinder somites of the gill-bearing region.

Whilst A. fluviatilis, A. leptodactylus, A. pachypus, and A. colchicus have three rudimentary pleurobranchiæ, A. pallipes has only two, the third most anterior rudiment having been reduced to a minute papilla, and A. torrentium has two without the least trace of the third.

The crayfishes which are used by students in University and college classes in this country are supplied by London agents, as a rule, who make a regular business of importing

[1] 'Mem. Mus. Comp. Zool.,' Harvard, x (4), 1885, and 'Proc. U. S. Nat. Mus.,' xx, pp. 643–694, 1898.

[2] 'Proc. Amer. Phil. Soc.,' xli, p. 286, 1902.

[3] The A. astacus (Linn.) of Faxon's later paper. Ortmann employs the generic name Potamobius in place of Astacus.

[4] Huxley's A. torrentium included this and the following species.

the various kinds. The native A. pallipes of the Thames was for many years used at Oxford, but within the last twenty years it has become rare in the Thames owing to a disease of the gills, and finer examples are now supplied by London dealers. These most frequently consist of French specimens, écrevisses à puttes rouges, the true Astacus fluviatilis, Fabr. On examining a specimen of the true A. fluviatilis in the Oxford laboratory, I observed a minute rudimentary gill in a position which appeared to correspond to the arthrodial membrane of Somite VII (that of the first pair of maxillipeds).

I give a more detailed account of this rudimentary gill below; here I wish to point out especially the very curious fact that this anterior rudimentary gill is not present in A. torrentium, A. pallipes, or A. leptodactylus, but it is present on both sides in every specimen of true A. fluviatilis which I have examined. These amount to about thirty, varying in size from $3\frac{1}{2}$ inches to $4\frac{4}{5}$ inches from the tip of the rostrum to the end of the telson. It thus becomes a specific character of A. fluviatilis, and the fact that it is not present in the smaller and larger species allied to A. fluviatilis goes some way towards explaining how it was that it escaped the observation of Professor Huxley, and that Oxford was for many years supplied with A. pallipes explains why it was not found in the Oxford laboratory before.

I have been enabled to examine a number of specimens of exotic species of Astacidæ belonging to the Natural History Museum, South Kensington, by the kindness of Professor Lankester, and have not discovered in them the new rudimentary anterior gill. However, in a male specimen of Astacus dauricus[1] from Corea, of length $5\frac{1}{2}$ centimetres from tip of rostrum to end of telson, on the right side and in exactly the same position as the new rudiment in A. fluviatilis there was a minute papilla, just visible to the naked eye, of length $\frac{1}{2}$ millimetre. This is the only specimen of A. dauricus which I have examined, and on the left side, which I looked at first, I could

[1] Specimens in the Museum collection are so labelled. More probably, however, they are A. (Cambaroides) similis, Koelbel. (W. T. C.)

find nothing, but as this part of the specimen was in a rather brittle condition I may have broken it away.

The other exotic specimens examined by me were:

Cambarus (rusticus ?) Astacinæ;

Parastacus pilimanus
Astacoides madagascarensis
Cheraps bicarinatus
Paranephrops planifrons
} Parastacinæ;

also Scyllarus latus, Madeira; Panulirus penicillatus, Gulf of Akaba; neither of which had any sign of the gill.

Description of the Rudimentary Posterior Arthrobranch on the Somite of the first Maxilliped in Astacus fluviatilis.

In the less well developed examples the gill appears as a small white filament resting on a white bulb or cushion (fig. 2) from which it depends outwards and downwards. In the better developed examples there are as many as seven filaments attached to a central stem depending from the cushion (figs. 1, 3, 4, 5, 6, 7). The sizes of cushion and gill vary from 2 mm. to $3\frac{1}{2}$ mm. gill and $1\frac{1}{2}$ mm. to 3 mm. cushion in crayfish of length $4\frac{1}{4}$ to $4\frac{4}{5}$ inches, and 2 mm. gill and 1 mm. to $1\frac{1}{2}$ mm. cushion in crayfish of length $3\frac{4}{5}$ to $3\frac{1}{2}$ inches. This bulb or cushion at the base of the gill is also present in the rudimentary pleurobranchiæ, but is nothing like so large in proportion to the filament. Minute hooked setæ are present on the cushion and sometimes on the stem of the gill (figs. 4, 5, 6, 7, *a*). The relative sizes of cushion and gill vary in different specimens. The position of the gill is shown in figs. 1 and 2; it is situated on the somite of the first maxilliped. The cushion is attached to, or rather springs from, the upper part of the edge of the lamina (fig. 1, *k*), which connects the epipodite (fig. 1, *g*) with the hard ridge (fig. 1, *e*); the cushion is also firmly attached to the ridge *e*, so that if

the first maxilliped be torn from the animal the cushion and gill stay behind. This position corresponds to that assigned by Claus[1] to the rudimentary gill on the first maxilliped in Penæus, as he objected to the two arthrobranchs of Huxley being classed together, and considered the posterior one as having a closer relation to the series of pleurobranchiæ. The epipodite passes posteriorly to the gill and touching it.

The amount of development of this gill, as with most rudimentary organs, is very variable, but it was fairly equally developed on the two sides of the animals I have examined (figs. 5 and 7, also 4 and 6, from same specimens); also it varies equally in development in males and females. In the better developed specimens in which there is a central stem the filaments of the gill are all developed on the outer side of this stem (figs. 1, 3, 4, 7). The filaments are frequently discoloured with brown patches.

According to Dr. Calman the only other Decapods known to possess branchiæ on the first thoracic somite[2] are Stenopus, some Penæidæ, and certain aberrant Thalassinidæ (Jaxea and Naushonia) which possess a minute arthrobranch on each side of that somite.

In Penæus the gill is less rudimentary than in A. fluviatilis, and rests on a fleshy lobe or cushion in the same position as that in Astacus, but which stands out straight from the body of the animal instead of lying flat against it as in A. fluviatilis. The filaments of the gill, of which there are many more than in A. fluviatilis, all spring from the cushion in a fan shape, not from a central stem as in A. fluviatilis.

As before mentioned, this gill is only found in A. fluviatilis, Stenopus, and some Penæidæ and Thalassinidæ; however, in A. dauricus there was the minute papilla on the right side of the specimen I examined, and there seem to be traces of the gill in some other of the allied forms which I examined.

[1] 'Arb. Zool. Inst. Wien,' tome 6, p. 46, 1886.

[2] Apart from the branchial filaments developed on the epipodite of the first maxilliped in many Parastacinæ.

In a specimen of Nephrops norvegicus, lent me by the British Museum, in exactly the same position as the cushion in A. fluviatilis is a partly calcified flap which hooks over the epipodite of the same somite, and apparently serves to prevent its coming forward. In Homarus vulgaris this hook is larger and easier to make out.

A specimen of Cambarus (rusticus?) male, from British North America, had in the same position a small hard knob; one of A. torrentium (male), from Bavaria, had a small hard cushion in the same position. Another of A. leptodactylus female, Asia Minor, also had a cushion in the same position.

According to W. Faxon "the gills of A. gambelii present the nearest approach to the primitive type of any living members of the genus Astacus," in that the three rudimentary pleurobranchiæ are jointed near their base and possess, the middle pair two short lateral branches, and the anterior and posterior pairs one short lateral branch, at the joints. Unless this species proves also to possess the new rudimentary arthrobranch, its gill formula must, however, be considered less primitive than that of A. fluviatilis.

In conclusion, I take the opportunity of thanking Professor Ray Lankester for kindly helping me to write this paper, and for enabling me to examine the specimens in the British Museum, and Dr. Calman for helping me in so doing, and for important assistance as to the crustacean gill generally.

Oxford, October, 1904.

EXPLANATION OF PLATES 23 & 24,

Illustrating Margery Moseley's paper, "On the Existence of an Anterior Rudimentary Gill in Astacus Fluviatilis."

PLATE 23.

Fig. 1.—Left anterior rudimentary gill in situ from a male, $3\frac{1}{2}$ inches in length (from tip of rostrum to end of telson). Magnified 35 diameters.

A. Cushion to which gill is attached. B. Stem of gill to which seven filaments are attached. C. Cut edge of epipodite of first maxilliped. D. Bulb to which is attached scaphognathite, which is not shown. E. Strongly calcified ridge, part of thoracic wall, representing part of fused epimera of anterior thoracic segments. F. Cut edge of thoracic wall, which here turns outwards to join lining of branchiostegite. G. The part of epipodite not cut off. H. Pivot, part of thoracic wall, to which is articulated the coxopodite of the third maxilliped. I. Boss which bears coxopoditic setæ, which are not shown. K. Outer edge of lamina, part of first maxilliped, connecting that limb with hard ridge (E), and bearing at its upper end cushion to which gill is attached.

PLATE 24.

FIG. 2.—Left anterior rudimentary gill in situ, showing adjoining thoracic wall and limbs. Magnified 6 diameters. *a*. Cushion to which gill with single filament is attached. *b*. Calcified ridge as *e* in Fig. 1. *c*. Cut edge of thoracic wall. *d* and *e*. Regions of thoracic wall, *f*. Strongly calcified ridge, to which is attached arthrodial membrane of third maxilliped. *g*. Scar left by posterior arthrobranch, cut off. *h*. Scar left by podobranch, cut off. *i*. Exopodite of third maxilliped. *j*. Boss which bears coxopoditic setæ. *k*. Protopodite of mandible. *l*. Basipodite and coxopodite of first maxilla at their region of attachment to body-wall. *m*. Bulb to which is attached scaphognathite, which is cut off. *n*. Exopodite of first maxilliped. *o*. Exopodite of second maxilliped. *p*. Stump of podobranch, cut off, of second maxilliped. *q*. Stump of arthrobranch, cut off, of second maxilliped. *r*. Scar left by anterior arthrobranch, cut off. *s*. Scar left by scaphognathite. *t*. Scar left by epipodite, cut off, of first maxilliped. *u*. Endopodite of second maxilliped. *v*. Endopodite of third maxilliped. *w*. Basipodite of third maxilliped. *x*. Endopodite of first maxilla.

Figs. 3, 4, 5, and 7 viewed under microscope by transmitted light with coverglass. (*a*) Minute hooked setæ.

FIG. 3.—Rudimentary gill plume from right side. Magnified 30 diameters.

FIG. 4.—Rudimentary gill plume from left side of male. Magnified 35 diameters.

FIG. 5.—Rudimentary gill plume from left side. Magnified 38 diameters.

FIG. 6.—Rudimentary gill plume from right side of same specimen as Fig. 4, viewed in drop of spirit without coverglass. Magnified 28 diameters.

FIG. 7.—Rudimentary gill plume from right side of same specimen as Fig. 5. Magnified 34 diameters.

M. Moseley, del

Huth Lith^r London

Fig 1

Fig. 2.

Fig. 6.

Fig. 3.

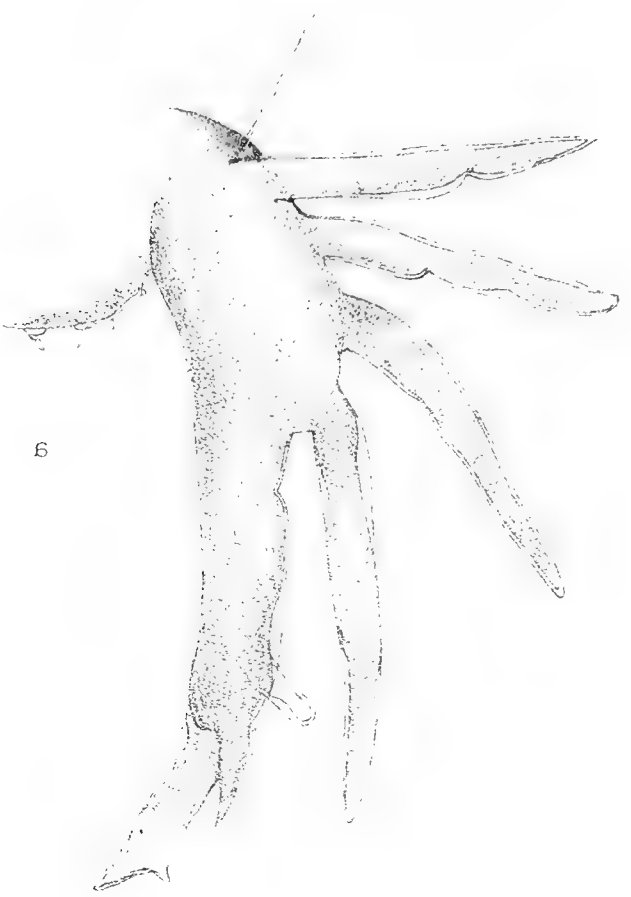


Fig. 4.

Fig. 7.

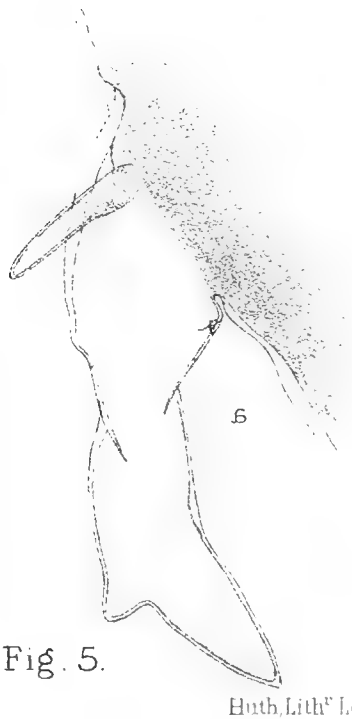


Fig. 5.

Huth, Lith^r London.

On the Development of Flagellated Organisms (Trypanosomes) from the Spleen Protozoic Parasites of Cachexial Fevers and Kala-Azar.

By

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(With Plate 25.)

The small oval parasites, known under the name of Leishman-Donovan bodies (although they appear to have been first found by D. D. Cunningham in Dehli boil) were described last year as occurring in the enlarged spleens of patients dying of chronic fever with marked cachexia by Leishman, who considered them to be degenerate trypanosomes, because he found somewhat similar bodies form with a large and a small chromatine mass in the spleens of rats which had died forty-eight hours before of trypanosomiasis due to the organisms of tsetse fly disease. Donovan, working in Madras, found similar bodies in blood obtained fresh from patients suffering from this fever, thus proving that those seen by Leishman were not degenerate trypanosomes, and Laveran, after examining Donovan's specimens, came to the conclusion that the parasite was a piroplasma. Ross, Nuttall, and Manson have all dissented from this view, and regard the organism as probably belonging to a new genus. Christophers suggests that it is a microsporidium.[1]

I have elsewhere shown that the parasite is to be found in

[1] Professor Ray Lankester, in the 'Quarterly Review,' July, 1904, expresses the view that Schaudinn's recently published researches, "On the Trypanosomes of the Blood of the Stone Owl," render it probable that Leishman's corpuscles, as well as those of Delhi sore, are stages in the life-history of a Trypanosoma.

the great majority of cachexial fevers with enlarged spleens occurring so commonly in Calcutta, and also in still larger numbers in all cases of active kala-azar, which, as I maintained in 1897, is nothing but a severe form of the disease hitherto known as "malarial cachexia," but for which I have suggested the more appropriate one of "cachexial fever" until further advances in our knowledge of the new parasite enabled a better one to be decided on. In the course of my recent investigations I tried various methods of studying the parasites outside the body, and eventually found one by which they could be kept alive for some days, during which they multiplied greatly, and in some instances developed new forms of considerable interest. The method by means of which these results have been obtained is an extremely simple one. The blood obtained by spleen puncture was immediately ejected into small sterile test-tubes containing a little sodium citrate to prevent the blood from coagulating, and these were then incubated at varying temperatures, portions of the culture being removed with a platinum loop from time to time for examination with the microscope. At blood heat I found the spleen parasites rapidly underwent degenerative changes, and after twenty-four hours most of them had disappeared and the remainder stained badly. As the presence of a macro- and a micro-nucleus in the spleen parasites pointed to their possible relationship with the flagellated class of protozoa, and it is known that trypanosomes live much longer out of the body at low temperatures than at blood heat, I next tried incubating the culture tubes in a cold incubator at 27° C., ice being used, as the laboratory temperature was several degrees above that point. At this temperature I found that the parasites lived for some days, retaining fully their staining properties. Further, in favourable cases, in which a large number of parasites were present in the blood when first obtained (which is only the case in about one fifth of those met with in Calcutta), it was soon evident that they were undergoing division and increasing very materially in numbers, for, instead of two or three in a field of an immer-

sion lens, as in the original specimens, as many as fifty or more were sometimes seen in the same area in those from the cultures. Moreover, divisional forms, which are rare in fresh spleen blood, appeared in very large numbers in the cultures after from one to three days, thus allowing the modes of division to be much more easily studied.

Divisional Forms without Development.

The divisional forms, which occur in great numbers in cultures at 27° C., are of two kinds. The first is a simple subdivision of the small oval parasites into two, both the macro- and the micro-nucleus first dividing, and then the body of the cell splitting into two, the cleavage beginning at one end, so that just before they separate they remain attached only by the other poles. This mode of division is illustrated in line I of the plate, figs. 1 to 4. These forms can be found in small numbers by long search in films of blood obtained by spleen puncture when numerous parasites are present, but they form only a very small proportion of the total number of organisms seen. On the other hand, in cultures they are present in very much larger numbers, several in various stages being often seen in a single field of the microscope.

The second mode of division is a multiple one, as shown in line I, figs. 5 to 8. The macro- and micro-nucleus divides a number of times, as in fig. 6, instead of only once, the outline of the cell becoming less definite, until eventually the appearance shown in fig. 7 is reached, in which a number of very small nuclei arranged in pairs of a small and a large kind enclosed in a zoogloœa-like material is seen. Next these enlarge gradually, and each pair becomes surrounded by a faint capsule, which becomes more and more distinct with the growth of each young form, until the characteristic groups of the oval bi-nucleated, fully-grown spleen parasites result, as shown in fig. 8 of line II of the plate, which are not very rarely seen in good specimens of spleen puncture blood.

Fig. 8 of line I shows a nearly full-sized group. All stages of these multiple divisional forms occur in large numbers in favourable cultures at 27° C., every stage being sometimes seen in a single field of the microscope. They are found most abundantly in a slimy material, which appears in the tubes after a day or two, and which stains rather like fibrin, but contains very few red corpuscles. This mode of division also takes place within the spleen during life, probably accounting for the greater number of the parasites, and the different stages can be seen in smears made from the organ shortly after death. The smallest multiple form is, however, very rarely seen in films of blood obtained by spleen puncture, probably because the cells, distended by a number of the larger forms, are more readily ruptured by the suction action of the syringe than are those containing the smaller forms. The formation of these multiple young forms in a zoogloœa-like material derived apparently from the protoplasm of the dividing parasite itself, and occurring in culture-tubes in which the blood-corpuscles have broken down, clearly proves that the parasites are not growing in the red corpuscles, and thus renders Laveran's contention that the parasites are piroplasma untenable.

At a temperature of 27° C. only the above-described forms were seen in large numbers. Noye's blood-agar culture medium was also tried without success. On next reducing the temperature of the cold incubator down to about 22° C. and making further cultures in a new series of cases of citrated spleen blood, further and more important changes were soon found.

Developmental Forms.

The first thing noticed was an enlargement of the small oval spleen parasites, affecting especially the macro-nucleus and the protoplasm of the cell, the micro-nucleus remaining unchanged. Then one day a culture of only twenty-four hours' growth, the fully developed flagellated forms shown

in figs. 8 to 12 of line XI of the plate, were suddenly encountered, together with the intermediate forms shown in the first seven figures of the same line. Since that time a number of cultures have been made and further intermediate forms have been met with, but in these it has taken three or four days before large flagellated forms were found, and the fully elongated trypanosoma-like forms of case 37 have not again been seen so perfectly. What the conditions were which favoured the full development in so short a time in that case I cannot say. The case was a more acute one than is often seen in Calcutta, but a second lot of spleen blood obtained a few days later failed to develop in the same way, so there must have been some other factor present. As in all my other successful cultures the steady development of the parasites day by day could readily be traced, it will be best to describe these changes in the order of their development. For the purpose of illustrating the progress of the evolution the forms seen each day in two cases have been drawn in the plate, each line representing one day's appearances.

Stage of Development after Twenty-four Hours. —At the end of one day at 22° C. an examination of the citrated blood shows the forms figured in lines III and VII of the plate, while lines II and VI show those seen in the spleen blood of the same cases before incubation. It will be seen from line III that at the end of one day the organisms have already increased considerably in size, while the macronucleus is also larger, this being a striking feature. On the other hand, the micro-nucleus has not altered, but still remains small and rod shaped. The forms shown in line VII also show that the macro-nucleus, in addition to being larger, is beginning to present a granular appearance, while it does not stain so darkly as in the original spleen parasites. Further, the protoplasm of the cell is also increasing in amount and now take on a bluish staining, and has a very finely granular appearance. These are the only changes met with as a rule on the first day.

Stage of Development after Forty-eight Hours.—By the end of the second day much more marked changes are met with, the principal forms of which are shown in lines IV and VIII of the Plate. In the first place there is a still further and very marked increase in the size of the organisms still affecting especially the macronucleus and the protoplasm, as in figs. 5 and 7 of line IV. Secondly, and of much greater interest, is the appearance of double forms, such as are not met with on the first day. These show every degree from apposition at one point of their circumference of two of the large oval forms, through closer degrees of contact up to nearly complete fusion of the two cells. At first I took these stages for a method of division, but as a further study showed that the latter developments into elongated and flagellated forms always takes place in pairs or rarely threes, I have come to the conclusion that these early double forms are really a kind of conjugation, such as is known to occur in other protozoa preparatory to the evolution of new stages in their life history. In favour of this view there is also the fact that the pairs of large oval organisms during the second and third days are found to be in contact with each other in very varying positions, and to present no regularity in this feature, as is the case with the small spleen forms undergoing fission shown in figs. 1 to 4 of line I of the Plate. Thus, while figs. 4 and 6 of line IV show contact of the sides of the oval bodies, figs. 5 and 7 of line V show apposition of the end of one to the side of the other, and similar variations are shown in the figures of line VIII.

In addition to the forms showing mere apposition, others show more or less complete degrees of fusion of two oval forms, as in figs. 1, 2, and 8 of line IV, the two macro- and micronuclei being each distinctly seen. Further, even on the second day, forms approximating to the next stage in the development of the organism may be found—namely, an elongation of the conjugating forms, as shown in figs. 1, 6, and 7 of line VIII,—but as a rule these do not appear in any numbers until the third day.

Stages of Development after Seventy-two Hours.—The third day is characterised by the elongation of the conjugating pairs of organisms, and the first appearance of flagellated forms, although sometimes the latter may not be found until the fourth day. The commonest appearance of these pyriform bodies is that shown in fig. 1 of line V, in which the macronuclei are seen in the thick ends of the organisms, while the micronuclei have passed to the thinner ends from which the flagella will eventually arise. In Case 58, from which the figures of lines II to V have been drawn, the culture-tube was unfortunately left out of the cold incubator for half an hour owing to an interruption in my work, and no further development occurred although the temperature of the laboratory was only 28° C. at the time ; so sensitive are the partially-developed forms to a rise of the thermometer. In Case 47 some early flagellated forms were found on the third day, as shown in figs. 4, 5, and 6 of line IX. In these only a single flagellum has yet developed although two of the forms are distinctly double ones, while some which appear to be single are really double ones lying on one side, for intermediate appearances showing the double nuclei partially obscuring each other in this manner have been met with. The remaining forms shown in line IX have all reached the elongated stage although still without flagella.

Stage of Development after Ninety-six Hours.—In the figures of line X are shown some of the flagellated forms found on the fourth day in Case 47, in addition to which there were much more numerous double pyriform organisms without flagella, for only a very small percentage of the conjugating forms eventually reach the flagellated stage under the artificial conditions of the cultures, which must be very far from being as favourable to the development of the organism as the natural conditions in which it takes place, whatever they may be. Nevertheless, the elongated flagellated forms have now been found in eight different cases, including two of kala-azar from Assam. In fig. 3 of line X the two flagellated bodies have apparently just separated.

Very occasionally groups of three instead of two organisms are found both in the early conjugating stage and in the later elongated and flagellated forms, as shown in fig. 2 of line X.

The Trypanosome-like Stage of Development.—From the forms so far described all that could safely be said is that flagellated organisms with an elongated body and micronucleus at the flagellated end have been obtained, but it could hardly be called a definite trypanosome. However, the forms shown in line XI of the plate go far to support the view that the organism is really a trypanosome, these having been found in a one day culture of Case 37, in which the conditions must have been in some unknown way much more favourable to the development of the organism than in the other cases. The forms shown in figs. 8 to 12 of line XI are precisely like the flagellated forms described above, except that they have elongated out to a much greater degree, so as to very closely resemble trypanosomes in everything except the absence of an undulating membrane, but this is known to be absent in very young trypanosomes, so that it would be expected to be the last feature to be developed in the growth of the organism from the plasmodial spleen form. The double forms shown in figs. 8, 10, and 12 of line XI are of great interest as an indication that these trypanosome-like forms have also developed in pairs, as in the more pyriform flagellated forms shown in line X. Further, fig. 9 of line XI was one of two precisely similar forms lying close together as if they had just separated, as in fig. 3 of line X. Moreover, the figs. 2, 3, and 4 of line XI are precisely similar in nature to the early stages of Cases 47 and 58 already described, from which they only differ in the greater elongation of 3 and 4. A possible explanation of the more typically trypanosome-like appearance of the flagellated forms of Case 37 is that as they developed within twenty-four hours, instead of only after three or four days as in the other cases, they must have found the blood in which they were growing much less altered than it is after several days' incubation in a test-

tube, and consequently, the conditions being less unnatural, their development has more nearly approached the typical form of trypanosomes.

Amœboid Forms.—The small flagellated forms represented in figs. 1, 5, 6, and 7 of line XI are also of great interest, for they correspond very closely with the forms of Trypanosoma Brucii described by Rose Bradford and Plimmer in the 'Quarterly Journal of Microscopical Science' of February, 1902, as "amœboid" stages, and found by them in the lungs of animals affected by tsetse fly disease. The origin of the flagella from the micronuclei is well seen in figs. 6 and 7 of this series, which I have only found in this case, although that shown in fig. 5 has been met with in others as well. As these very delicate organisms do not appear to form part of the regular cycle of development of the trypanosome stage from the spleen parasites, it appears to me to be possible that they may be a portion of the life-history of the parasite which is well fitted to live in the circulation, and which might conceivably be carried from one patient to another by the bites of flies and mosquitoes without undergoing any development within the insects, just as I showed in a previous paper the trypanosoma of surra may be carried from one animal to another by the bites of horse flies in a purely mechanical manner, an observation which has since been confirmed both in South America and in the Philippine Islands. In this connection it is worth while recalling the fact that when Indian cattle are inoculated with the surra trypanosoma they suffer from only a mild chronic form of the disease, and the trypanosomes are only found in their blood for a few days after a definite incubation period. Nevertheless, they every now and then get attacks of fever for many months afterwards (very like the repeated attacks in cachexial fever and kala-azar), but trypanosoma can no longer be found in their blood at such times by ordinary microscopical examination. Nevertheless, I found that if a little of their blood, taken during one of these periodical attacks of fever, is inoculated into a susceptible animal they

readily contract a fatal form of surra with innumerable trypanosoma in their blood. It is possible that a small amœboid stage of the parasite is the infective agent in such cases, and that in a similar way the infection of cachexial fever may be due to some such form carried from one person to another by the bites of flies and mosquitoes. The fact, which I pointed out some years ago in the case of kala-azar, that the infection is very largely a house one and always extremely localised (the movement of healthy people from an infected line to a new site half a mile or so away which I recommended having proved successful in preventing the spread of the disease), is in favour of such a mode of infection.

It is also worthy of note that the plasmodial form of T. Brucii described by Rose Bradford and Plimmer very closely resembles the parasites found in the spleen of these chronic fevers in man and the small multiple forms in my tubes; so that in this disease I have now obtained in cultures the plasmodial, amœboid, and flagellated forms found by those authors in a variety of animals after long study of the disease produced by the T. Brucii; a fact which can leave but little room for doubt that the human parasite belongs to the trypanosomes. Successful inoculation experiments are still wanting to prove this, all the animals I have tested—including tank fish (which are commonly infected with a sluggish, much curved, double S-shaped trypanosome) having proved insusceptible even when injected with cultures containing the large flagellated form of the parasite; but further work based on the knowledge of the true nature of the organism now available should lead in time to further elucidation of a disease which is certainly second to none in the frequency and seriousness of the illness it produces in many parts of India, and also appears to be widely distributed in other countries.

EXPLANATION OF PLATE 25,

Illustrating Mr. Leonard Rogers' paper "On the Development of Flagellated Organisms (Trypanosomes) from the Spleen Protozoic Parasites of Cachexial Fever and Kala-Azar."

All the drawings in this plate were made from the actual specimens by the Medical College artist, Behari Lal Das, as seen under a $\frac{1}{12}$ lens and a No. 4 ocular, the magnification being 925 diameters. The preparations were all stained with Romanosky's stain, used by Leishman's method.

Line I, Figures 1 to 4, show the simple method of division of the spleen parasites, and 5 to 8 the multiple form of division.

Line II shows the organisms present in a film made from freshly obtained spleen blood, Figure 8 representing a group of young parasites.

Line III shows the forms found after one day's incubation of the same blood, the parasites showing only enlargement.

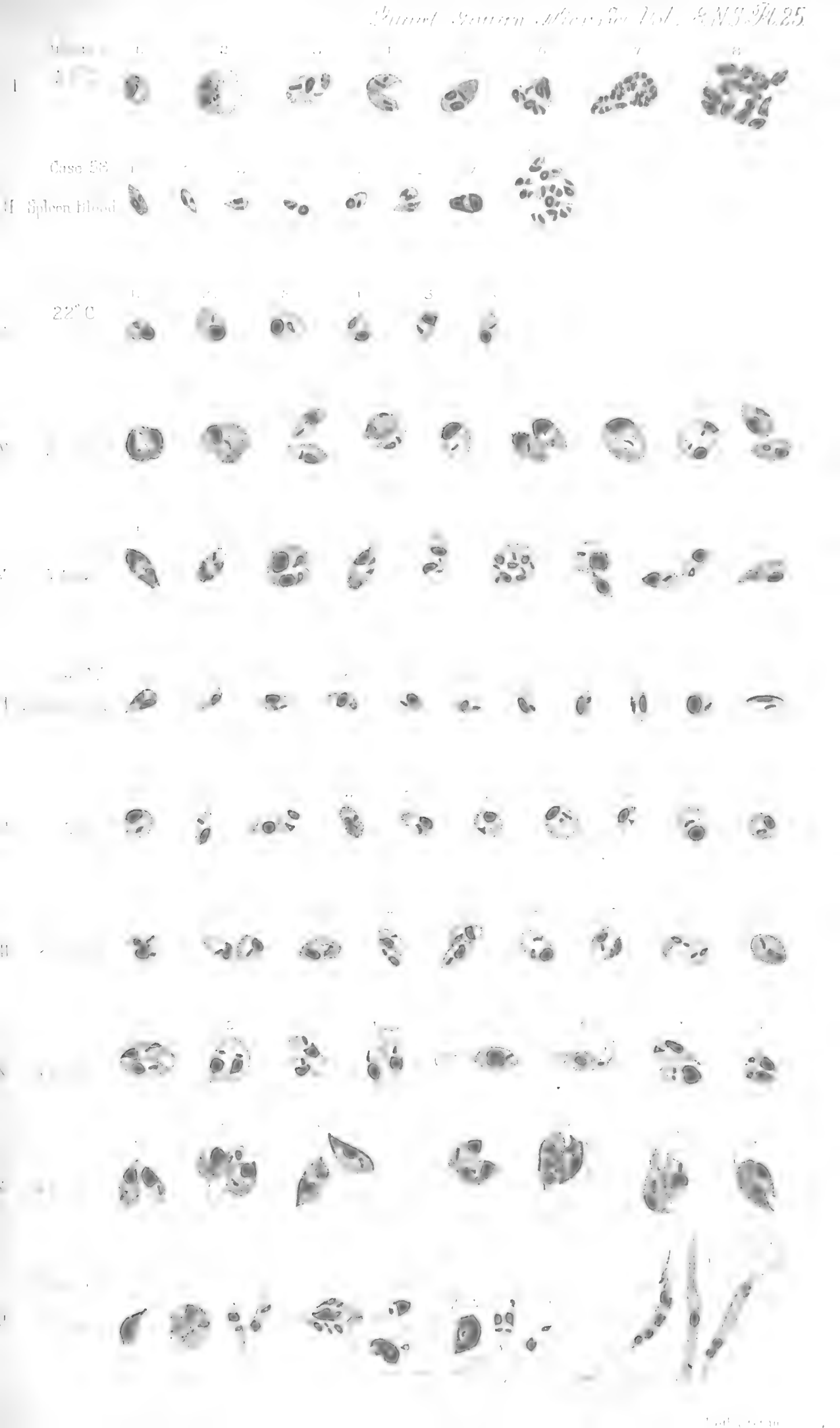
Line IV shows the same after two days, both single large oval forms and conjugating ones being represented.

Line V shows the same at the end of three days, both conjugating forms and elongated pairs being present.

Lines VI to IX show similar development day by day of Case 47, the early flagellated forms being seen in Figures 4 to 6 of Line IX.

Line X shows the large flagellated pairs, with the flagella arising from the ends containing the micronuclei.

Line XI shows all stages of the development from a one day culture of Case 37. Figures 8 to 12 represent the fully developed long trypanosome-forms with macro- and micro-nucleus, three of which still show the double form of the typical development. Figures 1, 5, 6, and 7 show the small flagellated amœboid forms resembling those found by Rose, Bradford, and Plimmer in Trypanosoma Brucii.


Case 58
Spleen Blood
22° C

The Epithelial Islets of the Pancreas in Teleostei.

By

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With Plates 26, 27, & 28.

Introductory and Historical.

The question of the anatomical and functional nature of the islet-like groups of cells occurring within the pancreas of vertebrate animals has been studied by a large number of investigators since attention was first directed to them by Langerhans in 1869. These inquiries have for the most part been confined to the higher vertebrates, and summaries of their results have already been given by other writers (Laguesse, 1894; Oppel, 1900). Notwithstanding the somewhat extensive literature of the subject, there is so much disagreement as to the real nature of these bodies that further inquiry was desirable. Oppel (**13**) wrote in 1900, "Was die Bedeutung der intertubularen Zellhaufen anlangt, so ist dieselbe, so viel auch daruber geschrieben wurde und so viele Ansichten auch daruber bestehen, noch nicht ganz erklart."

Although much has been written regarding these cell-

The present research has been carried out in several laboratories and upon material obtained from various sources. Cordial thanks are due to Prof. McIntosh, F.R.S., for the valuable privilege of the use of the Gatty Marine Laboratory, St. Andrews, and to Professors Heincke and Ehrenbaum, of Heligoland, not only for the free use of the laboratory there, with its abundant supply of material, but also for their friendly treatment during my stay in the institute under their charge. I acknowledge also the assistance of a grant from the Carnegie Trustees in defraying part of the cost of material and preparation for publication of this research.

groups, it appears that very little has been done in the investigation of the lower vertebrates. Indeed, until a short time ago, there appears to have been some doubt as to their existence in cold-blooded animals. According to Laguesse (7), "Les ilots de Langerhans paraissent constants chez les Mammiferes et les Oiseaux, leur existence est douteuse chez les Vertebres inferieurs. . . . Les auteurs ne les signalent pas en general chez les Vertebres inferieurs; Lewaschew les a cherches et ne les a jamais trouves chez les animaux a sang froid; Harris and Gow ne les ont pas vus chez les Reptiles, mais pretendent les apercevoir chez la grenouille; Von Ebner les y a decrits egalement. Enfin, quelques points des descriptions d'Ogata et de Platner pourraient s'interpreter en faveur de leur existence." No reference is made to their possible occurrence in fishes. Indeed, Harris and Gow, whom Laguesse quotes, expressly state that in consequence of their doubt as to the nature of the so-called pancreas in fishes they did not investigate the group. In the following year, however, Laguesse recorded the existence of cell-islets in the pancreas of Crenilabrus; in 1898, Massari (**12**) described them in the eel (Anguilla vulgaris); and in 1899 Diamare (**3**), in an important paper, established their occurrence in six different species of Teleostei, as well as in all the other vertebrate divisions. I shall have occasion later on to refer to the work and opinions of these writers.

In the investigation of which the present paper is a partial account attention has been limited to the bony fishes, of which about twenty-five different species have been studied. In general these islets are fewer in number and proportionately larger than in mammals. Owing to the diffuse condition of the pancreas in most Teleostei, they may, even when minute, be identified by the unaided eye. They are usually of a pale colour, and, being somewhat thicker, are seen distinctly against the more translucent sheet of the surrounding pancreatic alveoli. Wherever a careful search was made, either macro- or microscopically, these bodies were found, and hence it may reasonably be concluded they constitute a common feature of

this group. This is all the more probable since they appear to possess some functional activity.

As a result of pursuing specially the study of the conditions in bony fishes, I have, inter alia, discovered the existence of a "principal islet" (**15**). This has enabled me to offer a fresh suggestion as to the possible phylogenetic significance of these bodies in higher animals. I have, further, been able to confirm the opinion of Massari, Diamare, and others regarding them as ductless glands with internal secretory function, and also to test experimentally the theory that derangement of the function of these bodies leads to diabetes. A record of these experiments, which are still in progress, will appear later.

Names and Systematic Arrangement of the Species Examined.

Teleostei.

Physostomi:

Cyprinidæ—Cyprinus carpio.

Physoclysti:

Acanthopteri:

Scombriformes—Zeus faber, Agonus cataphractus, Lophius piscatorius, Cottus scorpius.

Gobiiformes—Cyclopterus lumpus, Callionymus lyra, Cyclogaster Montagui.

Bleniiformes—Anarrhichas lupus, Zoarces viviparus, Pholis gunnellus, Chirolophis galerita.

Anacanthini:

Gadidæ—Gadus virens, G. æglifinus, etc., Onos mustela.

Ophidiidæ—Ammodytes tobianus.

Pleuronectidæ — Hippoglossus vulgaris, Pleuronectes platessa, etc.

Lophobranchii:
Syngnathus acus, Nerophis æquoreus, Siphonostoma typhle.

General Relations of the Islets.

The following account indicates the general relations and macroscopic appearance of the bodies observed. In most instances, particularly in those cases where a "principal islet" is stated to exist, numerous specimens were examined.

Cyprinus carpio.—The islets observed in this species are among the smallest found. The pancreas is diffuse, and they appear in sections of it in different regions of the body-cavity. In some instances they lie alongside the zymogenous tissue, but in most instances they are surrounded by it, and do not possess a limiting capsule.

Zeus faber.—Here there exists a "principal islet," which, in specimens of about 25 cm., is as large as 5 mm. in length. There are also smaller forms in the neighbourhood of the pyloric cæca which may be dissected out, and also numerous microscopic ones within the intercæcal pancreatic masses. The principal islet lies within a small mass of zymogenous tissue, which is attached to the base of the gall-bladder (see Pl. 26, fig. 1). It and the smaller ones near the cæca were found ovoid in form. The intercæcal examples which are invested by more compact masses of zymogenous tissue, are rounded, oval, or irregular in outline. In serial sections they are seen to vary a good deal in this respect, owing to their being closely surrounded by the irregularly arranged pancreatic alveoli. The large forms have a more or less distinct limiting capsule; such a structure is not present in the smaller ones within the compact masses of the pancreas.

Agonus cataphractus.—In this species, occupying a position between the gall-bladder and the spleen, within a small mass of pancreatic tissue, is the principal islet. A similar body occurs close to the pyloric cæca. Both are contained within a firm connective-tissue capsule.

Lophius piscatorius.—In this species the number of islets which can be seen in a naked-eye examination is relatively large. The pancreas is diffuse, lying for the most part in the mesenteric area between the intestine anteriorly and the spleen. When this area is spread out (see Pl. 27, fig. 2), the islets, being opaque, may be easily observed. The "principal" lies a short distance in front of the spleen, several others are scattered between the cystic duct and the intestine. About half-way along this duct, between it and the intestine, there occurs with great frequency a fairly large islet, and, as already noted (**15**), several near the pylorus, amongst which is the second largest in size in this species. In appearance they are most frequently quite white, sometimes the minute vessels on their surface show as fine red streaks, and at others they are so distended with blood as to give the organ a dark ruddy hue. This variable appearance doubtless has some relation to the fact that retia mirabilia are numerous in and around the organ. The principal islet is in large specimens frequently of relatively great size. It is flattened, circular as a rule in outline, and ellipsoid in vertical section. In one case it measured 14 mm. in diameter, and about 5 mm. across its thickest part. It not infrequently in adults is as large as the supra-renal of a rabbit. The islet near the pylorus I have found 8 mm. in diameter. They are surrounded by a loose capsule of areolar tissue.

Cottus scorpius.—The pancreas in this species is in the form of narrow bands adhering to the intestine, and occupying the intercæcal spaces. One of these bands lies near the spleen. Immediately above this organ the principal islet may be seen with great distinctness even in small specimens as a pale, somewhat angular mass faintly streaked with blood-vessels. The portal vein passes close to it, and the main branches of the cœliaco-mesenteric artery pass ventralwards a little distance in front. In a specimen 22 cm. long the islet measured 3 mm. in diameter.

Cyclopterus lumpus.—Here there is a principal islet situated slightly anterior to the spleen. Its position and

relations are very similar to those described for Cottus scorpius. It is very pale and of relatively large size; in large specimens it is about 1 cm. in length. The amount of pancreatic tissue around it is very slight.

Callionymus lyra.—Several islets have been observed in this species, the largest—the principal—lying close to the portal vein on the right side of the fish where that vessel enters the liver. As in other cases, it is slightly anterior to the spleen, and in the same portion of the mesentery. The others are all in the same region, but lie nearer the intestine; they are whitish in appearance and very small.

Anarrhichas lupus.—Besides a principal islet, several others—never a large number—have been observed in the anterior region of the abdominal cavity. Except in the case of the principal, constantly occurring forms or large examples were not made out. The principal is usually ovoid in shape; in specimens of about 40 cm. its longest diameter is 9 mm. It is usually of a pale red colour, and lies in a thin sheet of pancreas in a portion of the mesentery well forward under the right lobe of the liver, and quite close to the mesenteric artery, from which vessel it is very easily injected.

Pholis gunnellus.—The situation of the principal islet in this species has already been indicated in my preliminary note. Further, in sections of the abdominal viscera in this region may be seen a fair-sized islet close to the intestine at the pylorus, a common position for these bodies.

Chirolophis galerita.—In a position very similar to that of the principal islet in Pholis there is an islet in this species. Only two examples were examined, and it was found in both. It is well forward under the right lobe of the liver, between it and the stomach, near the portal vein. It was found oval in form, enclosed in a firm capsule, and was easily separated from the surrounding tissue. In a specimen 15 cm. long it measured 2 mm.

Gadus virens.—Islets are situated in the intercæcal pancreatic tissue. They do not appear to be very numerous,

but some are of fairly large size; they are circular, elliptical, or irregular in outline. They do not possess a special limiting capsule, but are surrounded by the ordinary connective tissue of the pancreatic alveoli.

Gadus æglefinus.—The islets here occupy the same position as in the preceding species. Their relations to the zymogenous elements are also similar.

Cyclogaster Montagui.—Four specimens of this small species were examined, and in a position corresponding to that of the principal, an islet was in each case found. It is, of course, very minute, but may be found on the right side of the fish slightly anterior to the spleen and near to the pyloric cæca.

Zoarces viviparus.—In this species the principal islet occupies a position similar to that in Pholis gunnellus within the triangular area already referred to, which is slightly larger than in the related genus; the islet has a variable position, lying in some cases close to the hepatic artery and in others lower down in the angle between the mesenteric artery and the portal vein. There are present, in some instances at least, one or two smaller islets nearer the gut and within the area bounded by the vessels already named.

The pancreas is of the commonest type, viz. diffuse, and is sometimes greatly obscured by the presence of fatty tissue. Hence, although the islets are definitely separated from the zymogenous elements by a firm capsule, they may be more or less concealed by this tissue, and not so readily observed as in other instances. They are ovoid or spherical, and in medium sized adults the larger is about 2 mm. in length. The capsule is usually pigmented.

Onos mustela.—Islets exist within the pancreatic tissue which is found alongside the pyloric cæca. I noted in particular a large example of elongated irregular outline. The islets are in close relation to the ordinary pancreatic tissue, and do not have any special limiting capsule.

Ammodytes tobianus.—The pancreas here is of the

extended type, stretching the whole length of the intestine in two narrow bands, a condition which is common in small slender bodied fishes. A principal islet was not found by dissection, but on sectioning the entire viscera in the usual region of its occurrence several fairly large islets of irregular outline were found (fig. 6).

Hippoglossus vulgaris, Pleuronectes platessa, etc. —In the Pleuronectidæ examined the position of the principal islet is the same, and they may therefore in this section be referred to collectively. It is the same as in Zeus, viz. within a small pancreatic mass attached to the gall-bladder. In the larger forms, e. g. Hippoglossus, it is very apparent as an ovoid mass of a ruddy colour. Here the pancreatic investment is slight. In smaller forms the outline of the islet is not so apparent, being masked by the surrounding zymogenous tissue. Smaller islets exist in other parts of the pancreas; thus in both Hippoglossus and in Pleuronectes a fairly large one occurs with very great frequency, if not with absolute regularity, near the origin of the pyloric cæca.

Syngnathus acus, Nerophis æquoreus, Syphonostoma typhle.—In these Lophobranchs the pancreas consists mainly of two well-defined bands following the blood-vessels alongside the gut. On one of these portions where the portal vein crosses to the liver (the usual position, in fact) the principal islet occurs as a small, somewhat flattened, ovoid body, whitish in colour, slightly pigmented, and about 1 mm. in length in adult specimens. Its position in the three species is identical; it is found most readily by dissection from the right side, lying between the mesenteric artery and the portal vein (fig. 3).

In my previous paper I enumerated certain species in which a principal islet existed, and in the foregoing statement such particulars of its position in the several instances are given as will enable its occurrence to be verified. In the present paper additional examples are quoted, and it is more than probable that the list could be extended. For, after the general relations of the body became known, I failed to find

it in few fresh species, and these were of small size, where it is liable to be missed when sought for by macroscopic methods. It will be seen that the position is practically the same in all cases. It lies a short distance in front of the spleen in the mesenteric fold between the portal vein and the mesenteric artery. In a certain number this islet, though still in a position agreeing generally with the foregoing, has a relation which enables its situation to be even more exactly stated. It is in close proximity to the gall-bladder, and has the appearance of a compact nodule attached to its base or posterior wall.

In the following species—Agonus cataphractus, Lophius piscatorius, Pholis gunnellus, Anarrhichas lupus, Zoarces viviparus, Onos mustela, Ammodytes tobianus, Hippoglossus vulgaris, and Pleuronectes platessa—I found an islet at the pylorus. It was always smaller in size than the "principal." This is the one referred to in my preliminary note. Although I cannot at present say that it is constant, its presence here in so many different species is of interest and an indication at any rate that this is a common position. In one particular instance, Lophius piscatorius, I endeavoured to obtain some evidence on this question of constantly occurring islets. This species is the one in which I have found the largest number of these bodies. I examined many hundreds of specimens and I noted—

1. That the "principal," the largest islet (see Pl. 27, fig. 2), was present in every case.

2. That an islet at the pylorus (Pl. 27, fig. 2, *Is.* 1), and the islet marked "*Is.* 2" occurred very frequently, although they were not found in every case.

3. That there seemed to be considerable variation in the numbers of the others.

These facts are in complete accordance with the suggestion I have already made. Here constant and varying islets exist, whose relations may be compared to those of thyroid and accessory thyroids or spleen and accessory spleens.[1]

[1] In this connection it is worthy of note that I found accessory spleens of

The principal islet, where it exists, is on this view to be regarded as a distinct organ, the others as supplementary bodies of similar function. In those cases, viz. certain fishes and all higher animals, where it is presumably absent this organ in the course of phylogeny has disappeared and the supplementary bodies have increased in numbers and importance.

Histology.

The material made use of for histological purposes was fixed immediately after death, in either corrosive sublimate or Bles's fluid. After washing (in the case of the sublimate) and dehydration, the tissue was embedded in paraffin and cut serially with a Cambridge rocking microtome. The sections were stained with hæmatoxylin and eosin and examined under a Beck microscope possessing a $\frac{1}{14}$ oil immersion objective as well as lower powers.

In agreement with the results of other investigators, the tissue of these islets was found to stain a lighter tint than the surrounding pancreatic alveoli. Even when small they are very noticeable as paler areas in the pancreatic mass; they are frequently, however, more massive than the adjacent organ, from which they are definitely separated by a capsule. They are an epithelial tissue consisting of very small polyhedral or cylindrical cells well supplied with blood-capillaries. A common size of cell is about 10 μ across the narrow diameter.

In a number of instances there was noted a difference in the staining capacity of different areas within the islets. This feature has been noted by other observers, particularly Massari (**12**) and Diamare (**3**). The latter has interpreted it as indicating a difference in functional state of different parts of the islet; he regards such appearances as the accompaniment of different phases of the same kind of cell.

fairly frequent occurrence in Lophius. These appear to be common also in the skate, where two or three minute examples may be seen in a single fish.

Although some of the material examined by me might be interpreted similarly (e.g. in Zeus faber) in several instances, of which I give a detailed account, the contrast in size, form, structure, arrangement, and relation to the capillaries of the cells of the two regions of the islet, as well as their different staining capacities, appear to me so marked that I am not prepared to accept Diamare's explanation as satisfactory.

In the descriptions which follow I have not considered it necessary to detail the appearances in each of the species already enumerated; I have selected such as together illustrate fully the essential structure of these bodies as a whole.

Zeus faber.—In this species, apart from a slightly less intimate relation of islet and acini in the case of the principal as compared with the others, the histological structure of these bodies is similar in every respect. They are fairly numerous in the intercæcal pancreatic masses, where they lie close to the zymogenous tissue, and are without any special capsule. Some are of distinctly irregular outline. They consist of polyhedral cells, smaller than the cells of the pancreas; their walls are well defined, and the cytoplasm exhibits a delicate meshwork of fibrils. The nuclei are irregularly circular or oval, and show a large nucleolus which always stained a dark red. Chromatin net-knots were always observed, as also the nuclear membrane. Apart from size and the possession of a capsule of areolar tissue which may send in supporting partitions, the principal islet exhibits the same structure as the others. The pancreatic acini, however, in the examples sectioned, did not completely envelop the islet.

The differently staining patches already spoken of were usually observable in preparations of islets from this species. Examination with high powers showed that this difference is due mainly, though not altogether, to the staining capacity of the nuclei in the respective areas. The chromatin is more abundant in the nuclei which stain more darkly. At the same time, the cytoplasm of these cells appears to take

up a deeper tint than the cells of the lighter areas, though no structural differences were made out in this region of the cell. Capillaries are only fairly numerous in this type (fig. 4).

Pholis gunnellus.—The two islets referred to in an earlier part of this paper possess the same structure, except that the principal has a very definite capsule not apparent in the other, and its relation to the pancreas is less intimate. The structure is similar to that described for Zeus, but the cells are smaller and more cylindrical. Cell-walls are less easily seen, but are present. In parts the capillaries run in nearly parallel rows, and the cells are arranged in bands between. The cytoplasm shows a delicate network, which, in contrast to that visible in the neighbouring acini, is very fine. Differently staining areas, as noted in Zeus, were not observed.

Anarrhichas lupus.—The principal islet is a large body with a well-defined capsule which sends in numerous supporting partitions (fig. 5). It is surrounded by a very thin band of zymogenous tissue, from which portions penetrate within the islet. This feature is not uncommon in the case of large islets in fishes; Diamare noted it in some of the species examined by him, and I have met with several instances, as will be seen. The tissue of the islet proper presented similar features to that seen in Zeus. Light and dark areas were observable, and they were respectively traceable through series of sections. In the main the nuclei of the lightly staining areas were seen to be irregular in outline, while those of the dark areas were circular or oval. There was also a difference in size, the former being larger.

In Onos mustela a large islet of very irregular outline was examined. It occurred in a mass of pancreatic tissue adherent to the intestine at the pylorus. In some sections it appeared as two separate bodies, but examination of the series showed a connection. This islet had no limiting capsule, it was invested by the connective tissue of the

surrounding zymogenous elements, which were here massed and not spread out in a thin sheet as is more common in small fishes. Here, again, darkly and lightly staining patches of irregular outline were present, whose cells exhibited differences in arrangement and structure. No cell-walls were seen in the case of the darkly staining elements; but, from the very close arrangement of the nuclei, if walls were present the cells must have been of an elongated fusiform type. These cells are arranged in bands between the capillaries, and one could count half a dozen nuclei on an average in a row across a band between two capillaries. No network could be made out in the cytoplasm; the nuclear details were similar to those already given. These bands were not so definite in some parts as in others, and the arrangement resembles more the irregular grouping characteristic of the lighter staining areas of the islet. Although this was the case, it was noticeable that the nuclei of the irregularly arranged dark cells were more numerous than those of the light and also more crowded together. The lightly staining patches showed a more open appearance owing to the cells being larger; they were seen to be polyhedral in form. The nuclei did not differ much from those of the dark areas (fig. 13). In Cyclopterus lumpus the tissue of the principal islet is in every way similar to that here described. There is, however, a capsule around the body, outside of which a slight layer of pancreatic tissue is present.

Lophius piscatorius.—The islets are surrounded by a capsule of rather open areolar tissue. In those examined I observed no indication of an arrangement of the cells in bands as is apparent in many species; the tissue was quite uniform. Capillaries appeared abundant, and in the connective tissue around the islet as well as within it I noted the presence of retia mirabilia upon the vessels (fig. 10). As many as four were seen in a single section; the component vessels had distinct walls, and were united by a surrounding and interlacing connective tissue. The cells of the islet are of the usual polyhedral type, with cytoplasm

granular or fibrillate, the nuclei with distinct nucleolus and chromatin network. Distinctively dark and light areas were not observed.

Zoarces viviparus.—As may be seen from fig. 11, the relation between pancreas and principal islet is extremely slight. This is one of the cases where, were it not from the known relations as revealed in other species, it would be difficult to relate the body to its proper category. It is surrounded by a fairly thick capsule, upon which there is a deposition of pigment. I sectioned one throughout its whole length, and found that it was penetrated by no large blood-vessels, though capillaries were abundant. The greater part of the space within the capsule is occupied by bands of darkly staining cells. The parts between these bands, which wind irregularly, are occupied by cells which stain lightly and are of different form from the others. The columns of darkly staining cells are more richly supplied with capillaries than are the cells occupying the spaces between. The former are narrow, cylindrical, or fusiform, with very finely granular contents, and measure about 10 μ across the narrow diameter. The nucleus is oval, and stains very darkly, being filled with numerous minute chromatin granules. It frequently almost fills the width of the cell, and is about 9 μ. The cells of the lightly staining areas are irregularly polyhedral in form, their cytoplasm shows a network of fibrils rather than granules, while the nucleus has an irregular outline and contains fewer chromatin granules than that of the darkly staining form (fig. 12).

Ammodytes tobianus.—Besides small islets, two fairly large examples of irregular outline were found in this species. They were all completely enveloped in pancreatic tissue, and did not possess a limiting capsule. The capillaries were very abundant, so much so that in many parts they were separated from each other by the width of only a single cell. In consequence of this arrangement the cells appeared in columns in certain parts, they stained more darkly than the rest of the islet, and were of different form (fig. 6). The

contrast between the two was in fact marked. The darkly staining cells were columnar, with finely granular cytoplasm; nuclei almost uniformly oval with a distinct nucleolus and granular chromatin. The lighter staining cells were irregularly polygonal, their cytoplasm not so granular, and their nuclei of very irregular outline. A few of these are given in fig. 7. A frequent form is that with a deep cleft between two portions. There are fine fibrils of chromatin with net-knots and also distinct nucleolus visible. The contrast here is very different from the appearance in Zeus, and I do not think can reasonably be attributed to differences in functional state of the same kind of cell. They appear to me to constitute two interlacing tissues.

Pleuronectes platessa (figs. 8 and 9).—The principal islet lies within a small mass of pancreas which is attached to the gall-bladder. The greater part of this mass, which is that spoken of by Cole and Johnstone (1) as a "little nodular swelling," consists of islet; the zymogenous tissue forms a small envelope around it. There is a definite capsule, but I did not see any supporting trabeculæ. It is usually penetrated by zymogenous elements, and where this is so, connective tissue surrounds these and separates them from the islet tissue. This penetration by pancreatic tissue is a feature which has already been noted in other instances, and, as in those cases, it was here traceable as continuous with the same elements around the capsule. The components of the islet are very small cells richly supplied with capillaries. The capillaries are not equally distributed throughout, but are more abundant in the inner regions. In these parts the cells occur in columns or strands having a somewhat sinuous arrangement; they evidently in many instances surround and follow the course of the capillaries. These cells stain darkly. In the spaces between these winding strands, and also in other parts of the islet, where, as already indicated, the capillaries are not so numerous, cells, lightly staining, are massed. The arrangement is quite different, but besides a difference in staining capacity and relation to the capillaries,

certain structural differences were made out with high powers.

1. The form of the cell. In the darkly staining strands this was seen to be always more or less columnar, and was probably due to their position and arrangement between the capillaries. The cell-walls could not be made out clearly. The lightly staining cells are irregularly polyhedral, their walls could usually be traced with distinctness, and they are evidently larger than the columnar types.

2. The appearance of the cytoplasm. In the columnar types this appeared diffusely and finely granular. The light cells showed a fairly open network of fibrils.

3. The nuclei. In the columnar cells these appeared regularly ovoid or spherical, and smaller than those of the polyhedral type. These latter were very variable and very irregular in outline. Both kinds showed nucleoli and net-knots, the polyhedral cells showing these very clearly. Regarding those nuclear differences, I do not think they can be attributed to fixatives, since the irregular forms were found only among the polyhedral cells.

In Hippoglossus vulgaris the conditions are similar to those described for Pleuronectes.

In the Gadidæ only small islets were examined, and in these no special features were observed. The cells were all of one type, and similar to those of the lightly staining areas of those forms exhibiting two types. The islets were completely surrounded by pancreatic tissue, and no capsule was present.

Lophobranchii.—Examples of three different genera of this group were examined. A principal islet only was found, and this was sectioned in each case. There is a distinct limiting capsule, which may be pigmented, of a different tissue from that supporting the pancreatic acini. These latter are quite apart from the islet, and in fact are more closely associated with the mesenteric blood-vessels than with this body. Capillaries are numerous, but I did not find the cells arranged in columns around these, nor did I find two types of

cell. The elements were small, and resembled the lightly staining or polyhedral forms. They presented in their finer structure no features of a special nature.

The conditions observed in Anarrhicas, Onos, Cyclopterus, Zoarces, Ammodytes, Pleuronectes, and Hippoglossus, it will be noted, are suggestive of a double tissue within the islet. No such appearance is observable in the other forms examined. Massari describes a two-fold tissue in Anguilla, distinguishing the two kinds of cell as "chromatophile" and "achromatophile." Diamare has sought to refute this view, his interpretation, as already indicated, being that the differences seen are indications of different functional states in the two regions, and that there is one tissue only.

It is satisfactory to note that on the point at issue there is a remarkable agreement even in some matters of minute detail as to the actual conditions. Diamare's paper did not reach me until after my own observations were made, and although the species examined were not the same, and his methods of fixation and staining were different and more varied than mine, all the appearances noted by him are to be seen on my own preparations. The question is largely a matter of interpretation of results.

The facts are briefly these. Tracts of more or less columnar or fusiform cells wind through the islets, and around and between these are slightly larger polyhedral cells arranged in masses. The columns stain more darkly than the masses. Diamare speaks of tracts showing intermediate staining which force the suspicion that they do not represent two different categories of cells. I myself found islets in which the contrast in the two types was less marked than in others, but even here it is deserving of notice that the columns were always darker than the masses. If a difference of functional state be indicated by these appearances, we expect the columns will at some time show the lighter staining effect, and also that the polyhedral cells will be found in the darker phase. Such conditions were not found by me. Diamare, further, makes much of the fact that the one type

could be seen to be continuous with the other, sometimes a dark column merged with a light, or dark cells occurred on one side of a capillary and light ones on the other. I am not sure that there is much in this, but in any case he does not appear to have observed the contrast in grouping of the two sets as noted by me, e. g. in Pleuronectes, nor the relative distribution of the capillaries of the two regions. In this species I found the capillaries more abundant in the inner regions, and here the columnar cells were most noticeable, while the other type occupied along with the capillaries the spaces between and also the surrounding areas. In Zoarces the arrangement suggested by the grouping was that of columns of cells with an interstitial tissue. It is true that the columnar arrangement is due probably to the abundance of the capillaries, whose course the cells follow; but if they are all one tissue I have been unfortunate, as also has Massari, in seeing preparations which exhibited the columnar cells in one phase only, and that different from the rest of the islet. Diamare himself compares the appearance of an islet in Motella to the supra-renals of birds, where the cortical and medullary substances interlace.

I examined some very small islets in Ammodytes and Pleuronectes where there was only a limited number of cells visible in a section. They were so small that a difference of functional state between different parts was scarcely to be looked for, and yet the two types were apparent (fig. 6, *Sm. is, and Is.* 3).

Although I incline to the view that we have here two distinct tissues, from a consideration of the fact that in many species this double nature is not evident, I do not think they are likely to be of independent secreting function. The dark cells appear to stand in a relation intermediate to the capillaries on the one hand and the light-cells on the other. It may be that they regulate the supply to the capillaries of the substance secreted by the light-cells, or they may effect a final stage in its elaboration.

Relation of the Islets to the Pancreatic Acini.

From the foregoing account it will be seen that in bony fishes these islets, though undoubtedly existing under conditions similar to those met with in higher animals, also very commonly occur in distinctly less intimate morphological relation to the pancreatic alveoli. It will have been noted that various conditions have been observed, from that where the tissue of the islet stands in the same relation to the zymogenous elements as the separate alveoli of the latter do to each other (figs. 4 and 6) to cases where a thick investing capsule exists around a large islet with no alveoli in contact, and only a very few of these in the surrounding parts, e. g. Syngnathus (fig. 3). Indeed, my attention in the first instance was confined to these latter bodies in such fishes as Pholis gunnellus and Syngnathus acus, where the ordinary pancreatic tissue is in no more intimate relation to them than it is to the portal vein or mesenteric artery, along which vessels it extends as narrow bands. Accordingly I hesitated to relate these distinctly encapsuled and separate glands with the pancreatic "islet" of the usual type until I had found in various species bodies of identical structure in situations which left no further room for doubt. Amongst these encapsuled glands there is included the body already noted as a "principal islet." Diamare, without making any reference to the question of regular occurrence, describes this body in Orthagoriscus mola, Rhombus lævis, and Lophius piscatorius as a pancreatic islet. Indeed, no reasonable doubt can be raised as regards the homology of the principal islet.

A feature observed in the islets in Hippoglossus vulgaris, Pleuronectes platessa, and Anarrhichas lupus, viz. the penetration of these by zymogenous elements, had been previously noted by Diamare in other species. These elements may appear continuous with the pancreatic tissue outside, or as detached alveoli surrounded by the islet. In the latter

instances, however, they could be seen in serial sections to be continuous with the same tissue outside (figs. 5 and 8). I do not consider it a feature of any morphological importance. In all the cases where the peculiarity was noted the pancreas is of a very diffuse type, and in the spreading of its alveoli during development, as is well known, it may invade or become attached to other organs of independent function. A common feature is the close envelopment of the leading blood-vessels throughout the body-cavity by long strands of pancreatic tissue, and in several instances (Syngathus, Pleuronectes) such tissue accompanies the vessels within the liver, ramifying with these throughout the tissue of that organ.

Pancreatic elements penetrating the islets are supported by connective tissue, which is continuous with such tissue beyond and around the islet. On account of this Diamare has argued that the capsule is of the nature of interstitial tissue of the pancreas, and that here, owing to the larger development of these islets, it has assumed a capsular form; and he definitely opposes the interpretation that the tissue enclosed by the capsule is merely joined to the pancreas, and is not an inherent portion of it. The capsule, he says, in these cases is a "secondary" formation.

The view thus contested is, from evidence already partly submitted and partly to follow, one that I continue to hold. In the first instance we may recall the fact just referred to that a pancreas, intra-hepatic, exists; and if it can invade the tissue of an organ undoubtedly distinct and having embryonically a separate origin, there is no argument for identity in the fact that pancreas is found sometimes within another organ which has its rise from the same embryonic tissue,[1] and which we may assume is from the first in closer proximity. Further, I find the capsule is best developed where, owing to

[1] According to Laguesse (**9**) and, more recently, Pearce (**14**), they have the same embryonic origin. This does not affect the present argument, for we recall such facts as the origin of thymus and thyroid from branchial epithelium, and (according to S. Vincent) the medulla of the supra-renal from sympathetic elements.—J. R.

the extremely diffuse condition of the pancreas, interstitial tissue can scarcely be said to exist, the organ deriving support from the several other organs to which it is adherent, e. g. the larger blood-vessels (Lophobranchs). In fact, just in proportion as the pancreatic tissue has a more or less massive arrangement, the capsule is more or less indefinite. That is to say, the capsule tends to disappear where the form of the zymogenous tissue approaches most nearly the common form in higher animals. This is well seen in those fishes where the pyloric cæca have their interspaces filled with pancreas. The islets observed in such cases had no capsule. Where they have come to be enclosed within pancreatic elements, the necessity for a protecting capsule has ceased to exist.

In further support of the view which regards these islets as independent organs, two other points appear to me worth stating. In many fishes the peritoneal membrane and blood-vessels are pigmented, and in such cases so also is the capsule of these bodies, although I have never seen any pigment laid down within or upon pancreatic tissue. As examples may be quoted Pholis gunnellus, Zoarces viviparus. In this last I have found several islets within pigmented capsules in a single fish. The second point has reference to the intra-hepatic pancreas of certain fishes. If the islets are inherent portions of the pancreas in such fishes, related directly to its functions as a digestive gland, and, according to Jarotsky (**6**), who conducted an extensive series of experiments on white mice in dieting and fasting, "they probably supply a substance or substances representing a chemical stage of development of a ferment or substances necessary to the cells producing it," we would expect to find islets in the not inconsiderable part of the gland placed inside the liver. I have looked carefully for islets in this region in both Syngnathus acus and Pleuronectes platessa, and have found none.

The view I now bring forward may be stated as follows:—The conditions observed in various Teleostei force the conclusion that here "islet" and pancreas are distinct organs. In certain

genera, e.g. Lophius, Pholis, Zoarces, Syngnathus, the "islet" tissue has no more intimate relation to pancreas than to other neighbouring organs. Diamare, indeed, points out that the "islets" are glands of a more primitive type than the pancreas, which represents an advance in the evolution of organs. What he fails to appreciate is the fact that the more highly developed organ, in its most primitive state, is distinct from the still more primitive internal secreting gland. The compact pancreas, I consider, is a further development, in which the association of the two tissues is strengthened, so that they become virtually one organ, although there is no evidence but that they are still of independent function. This association is due to the fact that they arise from the same embryonic tissue. The results of Pearce (**14**) on the development of the islands in the human embryo are of interest, and their bearing on this point worth quoting. He does not agree with Laguesse and Renaut that the islands arise from "peculiar cells with rich eosinophilic protoplasm, comparable to the parietal or oxyntic cells of the gastric tubules." He finds that the pancreas develops as branching glandular processes, which become tubular later. The islets develop as side branches of these processes, and, from a careful study of the paper, I consider it clearly brought out that the island is formed from the "branching glandular process" before the remainder of it is transformed into acini. Thus the interesting point seems to be established that "island" is an earlier formation than acinus; that is, the phylogenetic order is paralleled in ontogeny.

Certain observers, investigating the pancreas of mammals, have concluded that the islets exhibit transitional forms indicating a change of islet tissue into gland lobuli. Lewaschew (**11**) claimed that irritation caused the groups to become more numerous and larger, and that various transitions became apparent. Statkewitsch (**16**) asserted that some of the lobuli of the pancreas underwent such changes during fasting that they assumed the islet form. On the other hand,

Diamare has, as the result of similar experiments, failed to find any appearance which might be taken as representing transitional forms, and his histological methods and results seem beyond reproach. Laguesse, who held the opinion that throughout life there was a repeated transformation of islet tissue into zymogenous and vice versâ, has (according to Pearce), in deference to Diamare's work, in large measure abandoned this view. I have never observed any appearances which might be regarded as transitional, but in any case the facts already adduced are entirely opposed to such interdependence as is here described. It is, indeed, quite possible that under such unnatural conditions as those of the experiments of Lewaschew or Statkewitsch the pancreatic lobuli underwent degeneration, or possibly reverted to the condition of the cellular "processes" of Pearce, which, although distinct in appearance from the islets, might well be mistaken for transitional forms.

The fact is not without interest that hitherto observers have failed to find anything like epithelial blood-islets within the pancreas in elasmobranchs. It is possible, assuming that they do not exist within this organ, that their function is carried out by certain of the other ductless glands in these fishes. In elasmobranchs both interrenal and supra-renal glands exist, while in teleosts adrenals, regarded as corresponding to the interrenals, are the only forms. May not one or other of the glands in the former group carry on the function of the missing "islets"?

The Function of the Islets.

Amongst the later investigators there appears to be agreement concerning the functional nature of these bodies. They are regarded as blood-glands with internal secretion. This is the opinion of Laguesse, who until recently held the somewhat peculiar view that they are alternately "endocrine islets" and "esocrine glands," the change being repeated during life. Other investigators, e. g. Dogiel (**2**), have held

them to be functionless effete portions of the pancreas, or embryonic remains. Others, again, regard them as contributing to the production of the pancreas secretion, e. g. Giannelli ed Giacomini (**4**) and Jarotsky, already quoted.

The facts as far as observed by me seem to point clearly to an internal secretory function. These bodies are ductless glands; they are all well supplied with capillaries, and in some cases these are very abundant. In some, structures for regulating the flow of blood through their tissues are present; and this, taken in conjunction with the different appearances met with in the cytoplasm and nuclei, leaves little doubt but that they are active organs. Whether two types of cell exist in certain instances or not, the irregularly polyhedral lightly staining forms occur in all. Reviewing these, it is noted that the nuclei occurred with regularly spherical or oval outline, and also very irregular in form. In the latter the chromatin was not so abundant; the cytoplasm, too, was more open and less granular. Such like differences Diamare also noted and correctly, I think, interpreted as indications of different functional states. They correspond, according to Baum ('Deutsch. Zeitschr. f. Thiermed. u. vergl. Pathol.' xii, 1886), with resting and active conditions respectively of gland-cells.

In a future paper I hope to give an account of certain experiments with extracts of these "islets," the results of which, as far as at present obtained, appear to indicate the presence in them of substances possessing some physiological activity.

Conclusions.

The occurrence of epithelial islets of the pancreas is widespread in Teleostei.

In many of these there is an encapsuled islet ("principal islet"), of relatively large size and of constant occurrence, whose relation to the pancreatic tissue is frequently extremely slight. In some species it was the only body of this nature found.

The smaller islets, which do not appear to be constant in number (Lophius), it is suggested, probably originated as "accessory bodies," but are now established as definite organs.

These islets are blood-glands which have entered into a secondary relation with the pancreas. This has been brought about in Teleostei mainly by the tendency of the diffuse pancreas to envelop or invade other tissues. In the case of these so-called islets in the compact pancreas of Teleostei, and also of higher animals, the closer relation is due to the common embryonic origin of the two tissues. Here the islets form a constituent part of the pancreas, although they maintain their function as an internal secretory gland. The primitive condition, however, is that seen in Teleostei with diffuse pancreas, where the islets are both morphologically and functionally distinct.

No evidence of transitional forms to support the view that the islets undergo metamorphosis into zymogenous tissue was found. The reputed changes of zymogenous elements to islet tissue are possibly degenerative, or regressive to the "cellular process" condition of the embryo.

From internal histological evidence, these bodies are probably functionally active. (Confirmatory of Diamare's work.)

References.

1. Cole and Johnstone.—"Pleuronectes," 'L.M.B.C. Memoirs,' 1901.

2. Dogiel, A. S.—"Zur frage über die Ausfürgänge des Pankreas des Menschen," 'Arch. f. Anat. u. Phys.,' Anat. Abt. Jahrg., s. 117—122, 1893.

3. Diamare, V.—"Studii comparativi sulle isole di Langerhans de pancreas," 'Intern. Monatsschr. f. Anat. u. Phys.,' Bd. xvi, pp. 155—209.

4. Giannelli ed Giacomini.—"Richerche istologiche sub tubo digerente dei Rettili," 'R. Accad. d. Fisiocrit. in Siena,' 1896.

5. Harris and Gow.—"Note upon one or two Points on the Comparative Histology of the Pancreas," 'Journ. of Anat. and Phys.,' vol. xv, pp. 349—360.

6. Jarotsky, A. J.—Über die Veränderungen in der grösse und im Bau der Pankreaszellen bei einigen Arten der Inanition," 'Arch. f. path. Anat. u. Phys.,' Bd. clvi, Heft 3, s. 409—450, 1899.

7. Laguesse, E.—"Structure et développement du pancréas d'après les travaux récents," 'Journ. de l'Anat. et de la Phys.,' vol. xxx, pp. 591—608 and 731—783.

8. Laguesse, E.—"Sur le Pancréas du Crénilabre, et particulièrement sur le pancréas intrahépatique," 'Rev. Biol. du Nord de la France,' vii, No. 9, pp. 343—360, 1895.

9. Laguesse, E.—"Sur la formation des îlots de Langerhans dans le pancréas," 'Compt. rend. de la Soc. biol.,' Annee 45, ser. 9, pp. 819, 820, 1893.

10. Langerhans, P.—"Beiträge zur mikroskopischen Anatomie der Bauchspeicheldrüse," 'Inaug. Diss.,' Berlin, 1869.

11. Lewaschew, S.—"Über eine eigentümliche Veränderung der Pankreaszellen warmblütiger Tiere bei starker Absonderungsthätigkeit der Drüse," 'Arch. f. mikr. Anat.,' Bd. xxvi, s. 453—485, 1886.

12. Massari.—"Sul pancreas di pesci," 'Rend. R. Accad. dei Lincei,' vol. vii, Fasc. 5, pp. 134—137, 1898.

13. Oppel, A.—'Lehrbuch der vergleichenden mikroskopischen Anatomie der Wirbeltiere,' Jena, 1900.

14. Pearce, R. M.—"The Development of the Islands of Langerhans in the Human Embryo," 'Amer. Journ. Anat.,' vol. ii. No. 4, Oct., 1903, pp. 445—455.

15. Rennie, J.—"On the Occurrence of a Principal Islet in the Pancreas of Teleostei," 'Journ. Anat. and Phys.,' vol. xxxvii, p. 375—378.

16. Statkewitsch, P.—"Über Veränderungen des Muskel- und Drüsengewebes sowie der Herzganglien beim Hungern," 'Arch. f. exper. Path. u. Pharm.,' Bd. xxxiii, pp. 415—461.

DESCRIPTION OF PLATES 26—28,

Illustrating Dr. John Rennie's paper on "The Epithelial Islets of the Pancreas in Teleostei."

References to all the Figures.

Art. Artery. *Cap.* Capsule. *ca.* Capillary. *c. t.* Connective tissue. *cy. d.* Cystic duct. *d. c.* Darkly staining cells. *g. b.* Gall-bladder. *Is.* Islet tissue. *Is.* 1 and *Is.* 2. Islets in Lophius referred to in text. *Is.* 3. Separated portion of large islet in Ammodytes. *Int.* Intestine. *l.* Liver. *l. c.* Lightly staining cells. *me. a.* Mesenteric artery. *p.* Pancreas. *p. d.* Pancreatic duct. *pa. is.* Pancreatic tissue within islet. *po. v.* Portal vein. *pr. is.* Principal islet.

py. c. Pyloric cæca. *re.* Rete mirabile. *sm. is.* Small islet in Ammodytes. *sp.* Spleen. *st.* Stomach. *v.* Vein.

PLATE 26.

Dissection of Zeus faber to show relation of principal islet to other organs.

PLATE 27.

Abdominal viscera of Lophius piscatorius, showing general distribution of the islets. The principal, which is always the largest, is seen directly anterior to the spleen.

PLATE 28.

FIG. 3.—Principal islet in Syngnathus acus. × about 50 times.

FIG. 4.—Intercæcal islet from Zeus faber. × 350. The centre portion throughout the series stained more darkly than the rest of the islet. Note the absence of a capsule.

FIG. 5.—Principal islet from Anarrhichas lupus. Here there is a slight penetration of its tissue by pancreas. The full thickness of the latter tissue in the proximity of the islet is shown. × 72 times.

FIG. 6.—Islet from Ammodytes tobianus. × 350. This islet shows well the relation to pancreas wherever the latter is at all massive. Dark and light cells are well contrasted. Capillaries are extremely abundant, but it should be noted that in this fish a similar appearance, in this respect, is seen in other organs, e. g. the liver. Besides the main islet, which in this section appears in two portions, there is a very small one to the right near a large vein. A large pancreatic duct is present.

FIG. 7.—Dark and light cells from the section in fig. 6. × 810. The nuclei (*n.*) in the light cells appear similiar to those seen by Diamare also, and described by him as "contorti."

FIG. 8.—Pyloric islet from Pleuronectes platessa. × 50. It shows areas of dark and light cells, and also a considerable amount of penetration of pancreas.

FIG. 9.—Portion of the principal islet of Pleuronectes, showing different appearances of the dark and light cells. × 810.

FIG. 10.—Rete mirabile from capsule of Lophius. × 810.

FIG. 11.—Principal islet from Zoarces viviparus. × 72.

FIG. 12.—(a) Dark and (b) light cells from islet in fig. 11. × 810.

FIG. 13.—Portion of islet from Onos mustela. × 810. Showing the contrast between the two types of cell in this species.

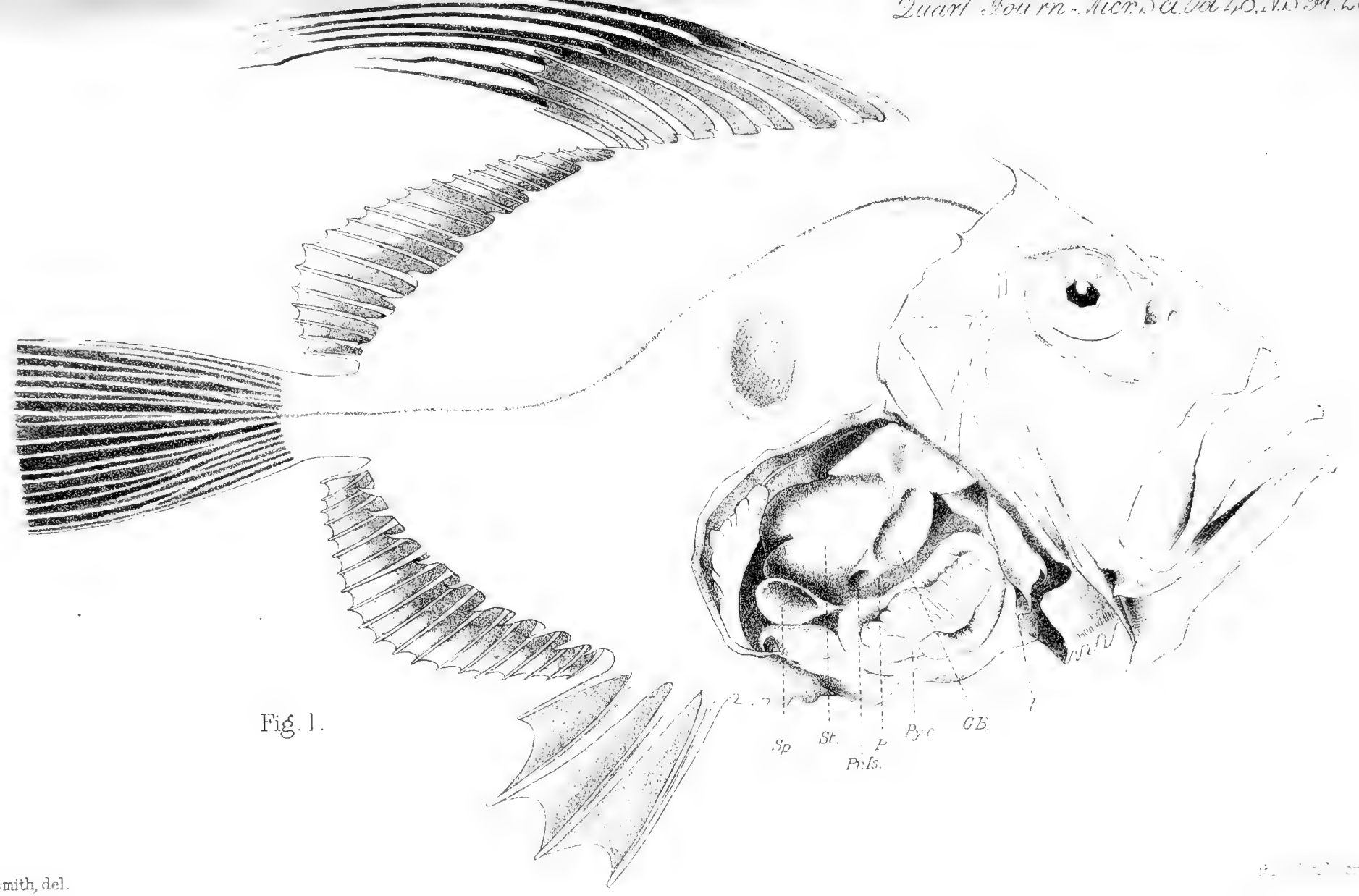

Fig. 1.

W. Smith, del.

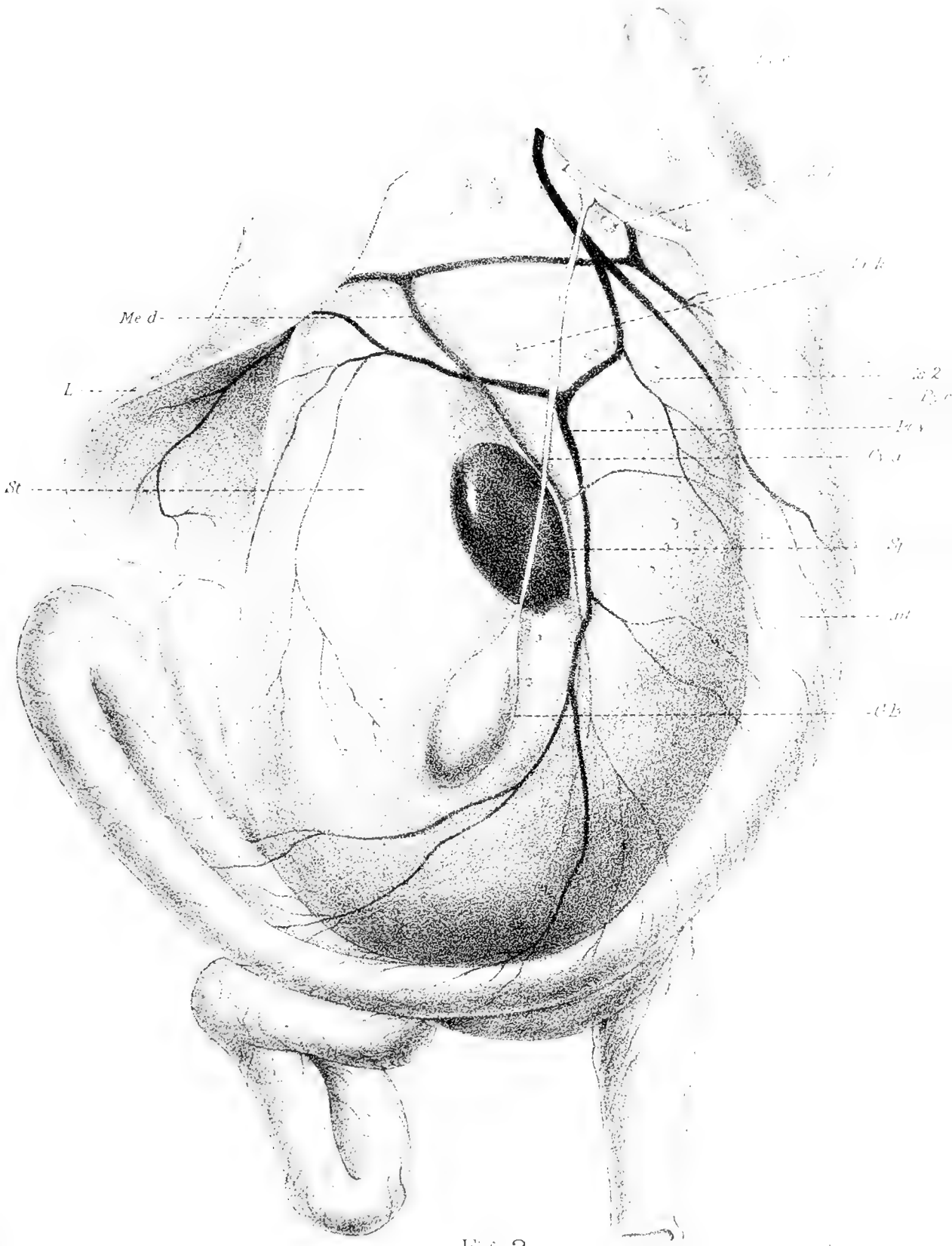

Fig. 2.

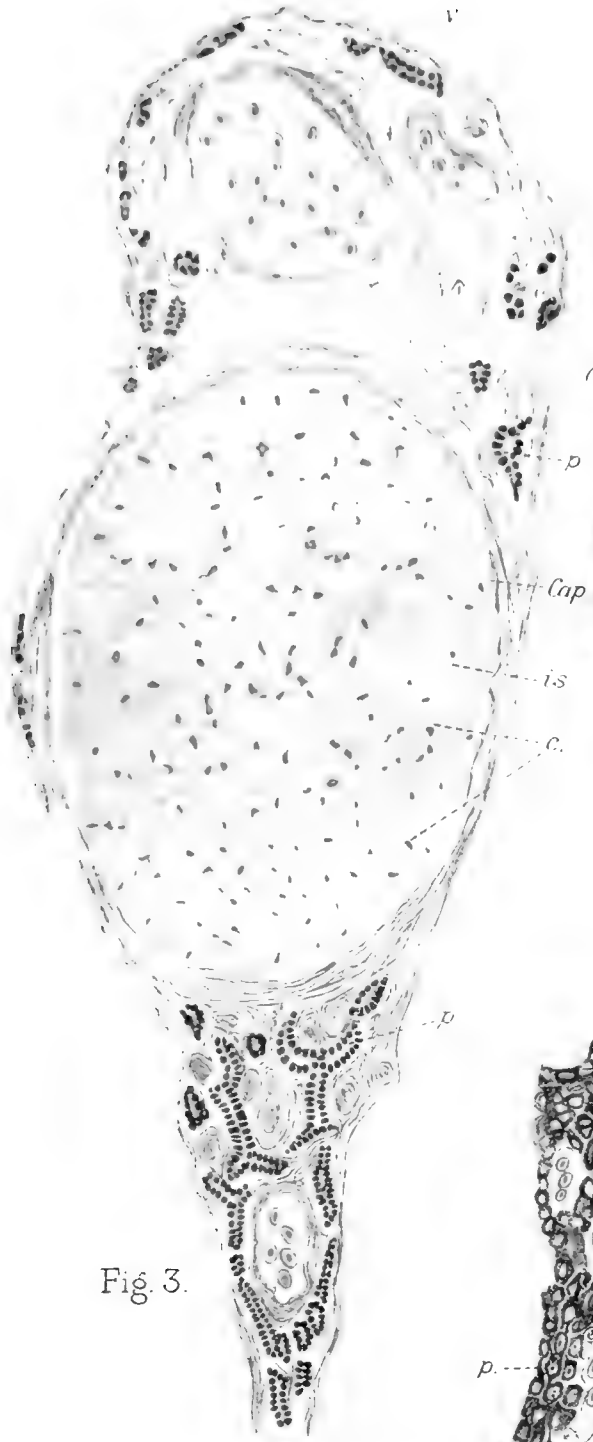

Fig. 3.

Fig. 4.

Fig. 13.

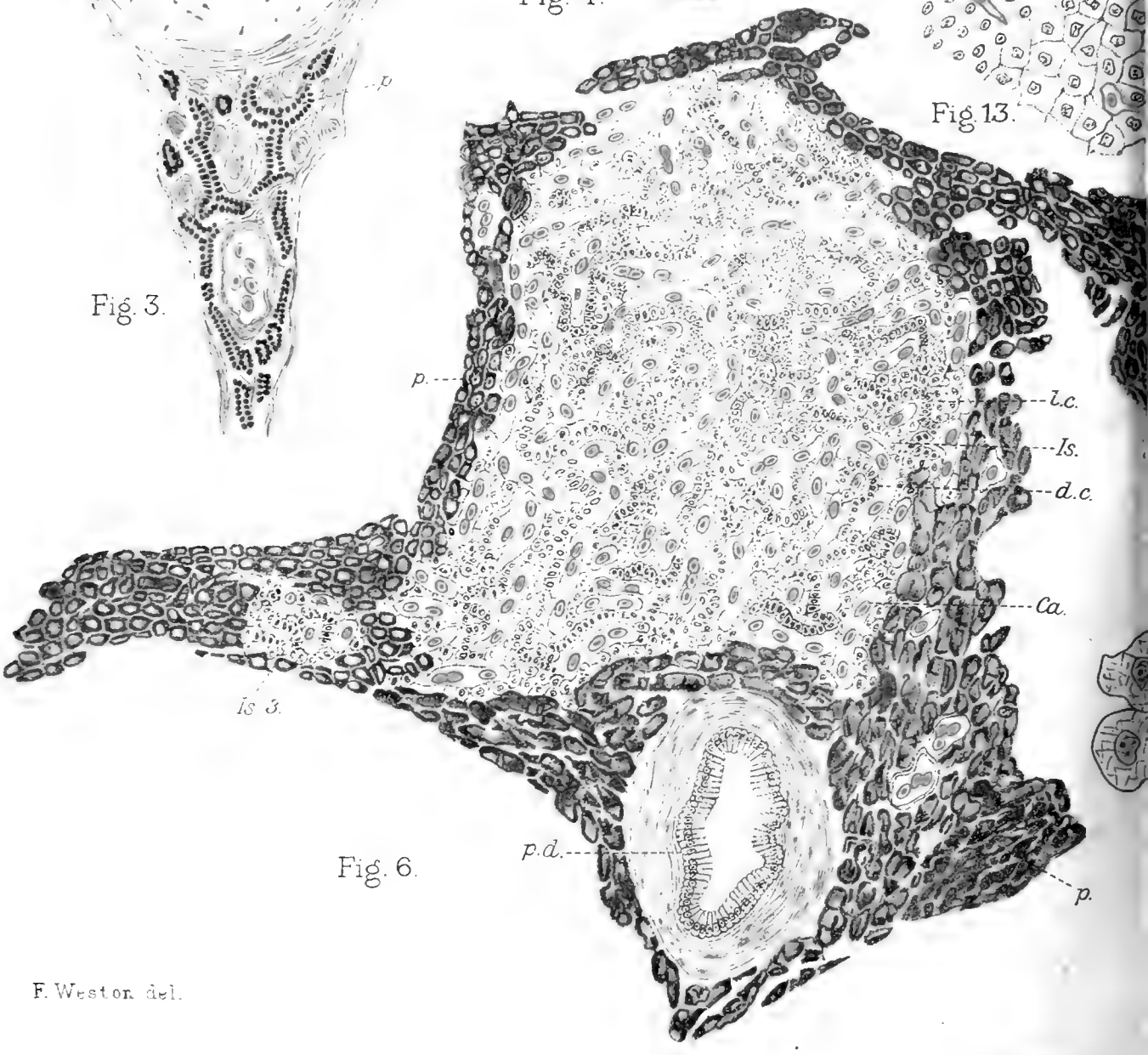

Fig. 6.

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Fig. 5

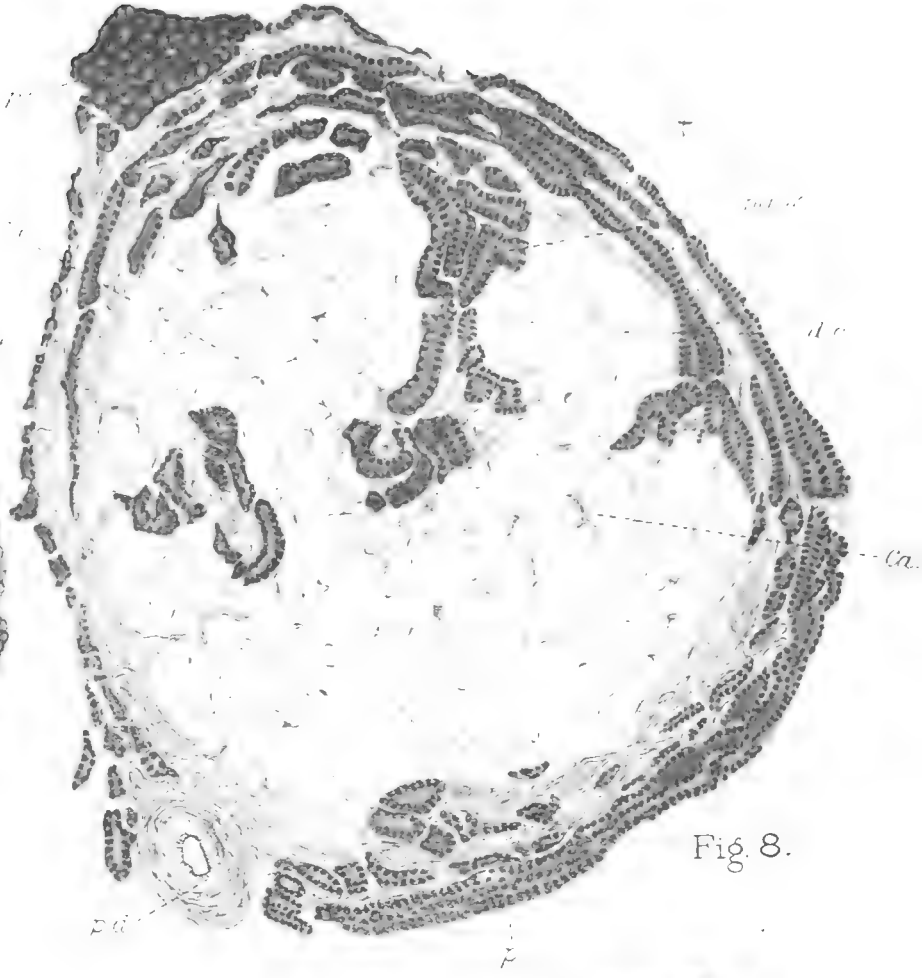


Fig. 8.

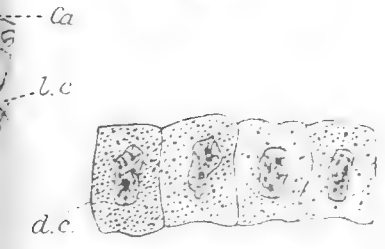
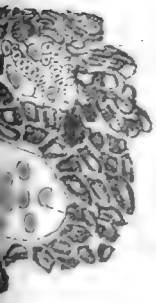


Fig 7.



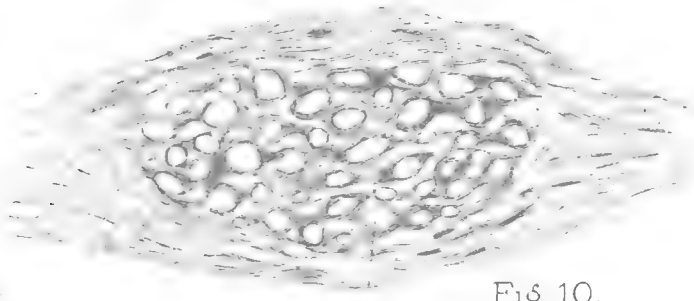


Fig 10.

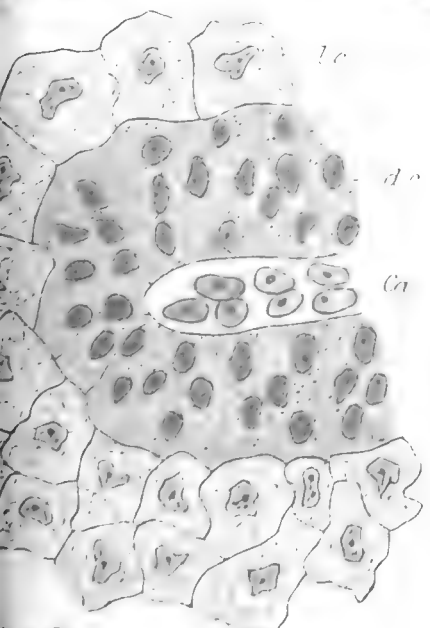


Fig 9

Fig 12.

Fig 11.

Huth, Lith. London

Observations on the Maturation and Fertilisation of the Egg of the Axolotl.

By

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Assistant to the Linacre Professor of Comparative Anatomy, Oxford.

With Plates 29—33.

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I. Introductory.

Eleven years have elapsed since the appearance of Rudolf Fick's memoir on the fertilization of the axolotl; yet, in spite of the host of authors who have since dealt with this, the earliest moment of development, his paper still stands out as one of the completest studies of the behaviour of the spermatozoon in the egg.

My own investigations were begun with no intention of controverting Fick's conclusions, but originated merely in the wish to demonstrate the process of fertilization to a class of students. In the result, however, I have found myself obliged to differ from my predecessor in one important particular, the origin of the cleavage centrosomes; and if in other respects I have succeeded in giving a more detailed description of the facts it must be set down simply to the modern improvements in our methods of research.

I have also included the phenomena of maturation in the field of my observations; but here I have been able to add but little to what the really admirable work of Carnoy and Le Brun has taught us of the polar divisions in many other Amphibia. I have indeed laboured under some difficulty here for want of sufficient material. Of all the females which I killed only one was found to have eggs in her oviducts. Of these only six, in the upper portion of the oviduct, exhibited stages of the first polar spindle; the remainder, a few in the middle region of the oviduct and a very large number in the uterus, were about to undergo the second maturation division. The rest of my material, which is fairly abundant, comprises eggs killed at various intervals after laying.

It is only quite recently, however, that I have been able to secure the most critical stages; I owe this to Professor Weldon's kindness in purchasing some fresh axolotls for my use. This will perhaps explain why my work, begun as long as three years ago, is only published now.

I have preserved the eggs in two mixtures: chromic ($\frac{1}{2}$ per cent.) ninety-five parts, glacial acetic five parts, and corrosive sublimate, with 5 per cent. to 10 per cent. acetic acid added. I tried a picro-corrosive mixture but found it useless.

The aceto-corrosive eggs have been stained in borax-carmine, followed by picro-indigo-carmine, and iron-hæmatoxylin; those preserved in chromic and acetic in gentian-violet, followed by eosin or orange, and in iron-hæmatoxylin. I have often unmounted preparations first stained in carmine or gentian and re-stained them in iron-hæmatoxylin.

The cutting of the eggs is a most formidable task, as any one who is acquainted with what Fick calls "die schwierige Technik der Amphibieneier-Untersuchung" will understand. Even with the very briefest sojourn in the water-bath the eggs become so brittle that it is impossible to cut them into continuous ribbons of unbroken sections. They must be cut on a Jung microtome with the knife oblique, and the block must be painted before each section is cut with a mixture of gum mastic and collodion dissolved in ether and absolute alcohol. The thickness of the sections was always 7·5 μ. The eggs were oriented by being placed, in a known position, in a square hole cut in an oblong slip of liver, and cemented down with albumen, which is then coagulated with alcohol. The liver, with the egg, can of course be cut in any desired plane.

I have ventured to add to the descriptive part of this paper, not only a critique of current theories of fertilization, but also an account of a few experiments I have made in the hope of throwing some light on the nature of the physical processes involved. In making these experiments I have had the advantage of the counsel and help of my friend Dr. Ramsden, of Pembroke College; I am under the greatest obligation to him for the assistance he has so generously afforded me.

I must not conclude this introductory chapter without attempting to define my attitude to the criticism which the botanist Alfred Fischer published two or three years ago

on the validity of our conceptions of cell structure and phenomena.

Fischer has shown that a structure can be given to solutions of proteids by precipitation with the ordinary fixing reagents, the structure being either granular or reticular, and from this he argues that much, if not all, of the structure observed in preparations is artifact and devoid of any natural existence whatever. Similar views were expressed about the same time by Hardy.

Doubtless there is much force in the criticism, but at the same time the thorough-going scepticism which Fischer would seem to advocate is surely a little exaggerated. For in the first place such structures as chromosomes, spindle, asters, centrosome have all been observed in the living cell. And in the second, when with the same reagent we find different appearances in successive stages of a process, then we are bound to assume that these differences are at least the outward and visible signs of a real series of changes. For example, I shall have to describe in the sequel the gradual formation of a system of vacuoles in the centre of the sperm sphere; these must be at least an indication of the local concentration of some watery substance, for on Fischer's own showing absorption of water precedes the formation of vacuoles in the artificial vacuolation of aleuron grains and such bodies which he produces by means of reagents. Nor is this all. If the different structures which we are asked to regard as artifacts form a regular series when placed in chronological order, is it not a little too much to expect us to believe that this artificial is merely parallel with, but in no way gives us a true representation of, that other unknown real series?

Without then going so far as to assert, what I suppose no one would maintain, that our reagents are absolutely infallible, I should certainly hold that such structures as those just referred to are faithfully preserved in our preparations. Fischer himself admits as much when he says "sind solche schon in der lebenden Zelle zu sehen so ist es zweifellos dass

sie auch vom Fixirungsmittel conservirt werden." Within this real structure alterations are undoubtedly produced (let me instance the frequently described microsomal structure of astral rays and the minute—reticular or alveolar—structure of cytoplasm); these must remain as a permanent source of difficulty which will always prevent us from deciding where nature leaves off and art begins. There are other cell structures again about which we should preserve a frankly open mind. I should certainly be prepared to admit for example that the achromatic reticulum of the nucleus was artificial.

Secondly Fischer has criticised the current views of the nature of the centrosome, aster, and spindle. This criticism falls into two parts; the first is an attack on the iron-hæmatoxylin method as diagnostic of the centrosome and centriole, the second is a theory of the formation of centrosomes and asters. The centrosome is regarded as produced through a precipitation of the albumins of the cell by nucleic acid, the nucleus opening for the purpose at the poles. The asters are also looked upon as precipitation products. Fischer has shown that a radial structure can be artificially made in two ways. In the first, which he terms "Fremdstrahlung," elder pith cells are injected with solutions of proteid and then fixed. Asters are found in the cells, bnt only when some small nodule is present to form a centre for the radiations. In the second method—"Selbststrahlung"—the rays are formed in a proteid solution about a crystal of sublimate or a drop of osmic exuding from a capillary tube. He suggests that in the living cells asters originate around the centrosome by one or other of these processes. In the first case the precipitating reagent is either the nucleic acid of the nucleus or the fixative employed; in the second it is the centrosome itself. Further, centrosome, aster, and spindle (formed by the conjunction of two asters) are looked upon as entirely passive, mere incidental accompaniments of the activities of the cell; for the movements of the chromosomes are attributed by Fischer to the ordinary streaming and growth motions of the cytoplasm.

The first part of this criticism has already been met by Boveri (1901), and I can do no better than fully endorse his reply. While admitting fully that many particles besides the centrosomes will stain in this way, and that many bodies which have been described as centrosomes, even at the poles of the spindle, may be the artificial products of "concentrische Entfärbung," he justly points out that two such bodies lying in a sphere, or one lying excentrically, cannot be thus accounted for. Moreover the centrosome, if not actually visible intra vitam, may often be seen in an unstained preparation.

The second part contains what I believe is a valuable contribution to the theory of the origin of both centrosome and aster, of the former through precipitation by nucleic acid, of the latter by a process of "Selbststrahlung" about the centrosome so produced. The conclusion drawn is, however, wholly unwarrantable, and would never have been adopted if, as Boveri points out, Fischer had kept the hard facts of cytology in sight, instead of deliberately ignoring the gradual cycle of changes which these cell organs undoubtedly pass through.

II. Descriptive.

A. Maturation.

1. First polar division.

(a) Metaphase.—In my earliest stage the spindle is fully formed, and is at the surface (fig. 1); its direction is either radial or slightly oblique. The spindle is closely surrounded by yolk-granules and pigment, and consists of wavy, frequently anastomosing fibrillæ. The appearance is not inconsistent with the view that we have here to do with elongated alveoli. Some of the spindle-fibres are united in definite bundles, and to some of these bundles the chromosomes are attached. Almost all the fibres pass continuously from one pole to the other, but at the outer end of the spindle

immediately below the surface, there are a few fibres radiating between the yoke-granules. These "mantle" fibres are the only representatives of an aster.

At the outer pole the fibres appear all to converge in a single dense mass, but at the inner end their behaviour varies in different preparations. In some cases this end of the spindle is also unipolar, but in other cases, as in that figured, the fibres undoubtedly converge to two separate points.

There is no trace of any centrosome at either spindle pole except the mass formed by the convergence of the fibres.

The chromosomes at this stage have the form of rings, which by being indented at four places assume the shape of a cross. The cross is so placed on the spindle that two arms —those by which it is attached to the fibres—are parallel to the spindle-axis, while the remaining two are either in or parallel to the equatorial plane, and therefore at right angles to the first two. These equatorial arms, however, do not lie in the same plane as the two meridional arms, but project outwards, making an angle with one another. Each such cruciform ring is in reality composed of two chromosomes, the extremities of which can be distinctly seen at the ends of the equatorial arms of the cross. These extremities are often twisted over one another, as indicated in the figure.

Though the above description may be taken as appropriate to a typical chromosome of this stage, many of these bodies are exceedingly irregular in form, twisted and contorted into many curious shapes. Such irregularities in the shape of the chromosomes in the first maturation spindle have been described by many authors, notably by Griffin for Thalassema, as well as by Carnoy and Le Brun for the Amphibia.

The chromosomes do not all lie in the equatorial plane, and are not confined to the outer surface of the spindle. They are scattered irregularly through it and at different levels. In the spindle, therefore, the fibres—or rather the fibre-bundles —attached to the chromosomes are mingled with those which pass from pole to pole, and the spindle is "mixed" according to Meves' (1896, 1898) nomenclature.

(b) Telophase (fig. 2).—The next stage I have is a telophase. The spindle consists of wavy bipolar fibres, but no bundles are to be seen. The chromosomes are united at each pole into an irregular, thick, annular skein; at the outer end the surface is raised up into a little flat disc with a homogeneous border. Later, this flat disc is constricted off as the first polar body, and found united only by a narrow stalk to the egg, and lying in a slight depression at the surface of the latter (fig. 3).

In the polar body the chromosomes are not yet distinct, as they will be later; there are also present pigment and yolk-granules. The stalk is fibrillated, the fibrillæ thickened to form "intermediate bodies" ("Zwischenkörper" of Flemming). The stalk contains a few pigment-granules.

In the egg the chromatin skein is resolved into chromosomes, which are V-shaped, aggregated by their apices, and lie in a clear area devoid of yolk-granules.

2. Second polar division.

(a) It is apparently from, or in, this clear area that the second polar spindle is formed, for a little later the chromosomes—which have meanwhile split longitudinally—are seen lying in an elongated area, which is distinctly fibrillated, and occupies a tangential position (fig. 4).

In the first polar body the chromosomes have simultaneously undergone longitudinal fission.

In one other preparation that I have the second polar spindle occupies a similar position, but the fibres are much more evident, and there seems to be a distinction between them, some being arranged in bundles and attached to chromosomes, others passing continuously from one end of the spindle to the other.

(b) Metaphase.—In describing the next stage in the formation of the second polar spindle I must distinguish between two lots of eggs; one lot was obtained from the oviduct and uterus, the second comprises freshly-laid ova.

To begin with the second, in all these ova the spindle is found in a radial or nearly radial position (fig. 5). It consists of outer and inner fibres; the former radiate out amongst yolk-granules and pigment, and lose themselves in the general cytoplasm; the fibres from opposite poles do not cross, but are diverted into the equatorial plane. They are to be regarded as astral rays. The inner fibres pass from pole to pole, are wavy, and frequently meet; certain of them are gathered together into bundles, and to these bundles the apices of the chromosomes are attached. Towards the poles the constituent fibres of the bundles again separate from one another and mingle with the general fibres of the spindle. If we examine a transverse section of such a spindle we find a polygonal meshwork thickened at the nodes; in addition, the fibre-bundles just described are seen occupying each the centre of a system of triangular areas. The whole appearance—as seen in both longitudinal and transverse section—is therefore quite consistent with the supposition that we are here dealing with elongated alveoli (I do not use the word with the whole of Bütschli's connotation), the fibres in that case being merely the optical sections of the inter-alveolar lamellæ.

At the outer pole of the spindle is a slight depression in the surface of the egg.

At both ends of the spindle the fibres converge to a dense granular mass, somewhat flattened in the direction of the spindle-axis, which may perhaps be regarded as a centrosome; but I am unable to state anything of its origin, and later it certainly disappears.

The chromosomes in the spindles are V-shaped, moniliform, and paired; they lie in the equatorial plane with their apices pointing inwards; they are not disposed in a regular ring, but some are nearer to, some further from, the spindle-axis. We have, therefore, here again a "mixed" spindle in Meves' sense.

In the other lot of eggs—that taken from the middle of the oviduct and from the uterus—the spindles are also radial,

or nearly so, and do not differ in any respect from those just described except that the outer end projects slightly from the surface of the egg (figs. 6 *a* and 6 *b*). The chromosomes, however, are beginning to diverge by their apices, and we can see in many—though not, I think, in every case—that these divergent points are still connected by a fine, frequently twisted thread (the connecting thread, or "Verbindungsfaden"). Further, the pairs of chromosomes are not placed so regularly in the equatorial plane, but many are scattered over the spindle.

From this one might argue that we are dealing here with a late prophase of mitosis, and this opinion is certainly strengthened by the fact that the ova in question were obtained from the middle part of an oviduct in the upper portion of which only stages of the first polar division were found. On the other hand, the commencing divergence of the chromosomes and the protrusion of the outer end of the spindle above the surface of the egg inclines me to the belief —though I cannot express a very positive opinion—that these spindles are in reality in the condition of the early anaphase. As a possible explanation of the irregular position of the chromosomes in the spindle, I may add that it is not unknown —a case is described by Boveri (1888), for example, in the egg of Ascaris—for both chromosomes of a pair to pass to one pole.

(c) Anaphase (figs. 7 *a* and 7 *b*).—In the later anaphase the daughter chromosomes pass in the ordinary way to the opposite poles, where their apices converge. Between them the general fibres of the spindle are clearly apparent; the fibre-bundles to which the chromosomes were attached can, however, no longer be distinguished. The external fibres have the same relations as in the previous stage.

The outer pole of the spindle is occupied by a dense hyaline mass, which passes together with some of the superficial pigment of the egg into the small projecting disc which marks the first appearance of the second polar body.

The second polar body, when fully formed (fig. 8), is a

slightly flattened, rounded mass, though much less flattened and much smaller than the first polar body. Like the latter it contains some pigment and yolk-granules. The narrow stalk by which it is connected to the egg contains the remains of the spindle fibres, but I have not observed any thickenings of these which could be identified as "Zwischenkörper." The chromosomes retain for a time the arrangement described in the last stage.

The second polar body is formed below or near the depression in which the first is lodged. It protrudes a little above the surface of the egg; the vitelline membrane is correspondingly pushed out.

3. Further history of the polar bodies.

In the first polar body the V-shaped chromosomes are united in pairs by their apices. At first they are closely grouped together, but later they become scattered, and each pair assumes a cruciform shape (fig. 9). It is now impossible to decide which of the four arms of the cross belong to which of the two constituent chromosomes, for all four arms are equally separated by constrictions from one another at the point of union. The surface of the chromosomes is produced at intervals into little tooth-like projections.

In one case only have I observed the reconstitution of a nucleus in the first polar body (fig. 10). The chromosomes are still distinct and still in pairs, but they lie in a circumscribed oval area which seems to contain an achromatic reticulum, staining dissimilarly to the cytoplasm. I ought to say, perhaps, that there is no doubt that this is a first and not a second polar body, for a second polar spindle is present in the same egg. At the same time it is possible that the cell just described is one of the two products of the division of the first polar body; its small size is in favour of this view. Fick saw one case of such division.

The first polar body always contains some pigment and yolk-granules; the latter tend to become aggregated into

irregular clumps. The polar body is in a slight depression at the surface of the egg. It persists for some time and may be found throughout the earlier stages of fertilization.

The second polar body also persists for a considerable time. Like the first it contains pigment and agglomerated yoke-granules. In it, however, the nucleus is very frequently reconstituted. A clear vacuole is formed round the chromosomes (figs. 11 and 12); these send out little processes towards the wall of this vacuole (fig. 13), which thus forms the nuclear membrane, and to one another. The chromosomes then break up into irregular coarse fragments (fig. 14); but I have never observed the formation of a completely reticular nucleus. These changes in the nucleus of the second polar body do not necessarily keep pace with the similar changes in the chromosomes which remain in the egg.

4. The direction of division of the chromosomes.

It is perfectly clear that in the second polar spindle the chromosomes are divided longitudinally, that is quantitatively in Weismann's sense. But in the case of the first maturation division I have not the material for deciding this point. The chromosomes are placed on the spindle in the form of rings, broken into two half-rings at the equator. This arrangement certainly reminds one at first sight very strongly of the heterotypical spindles of the Salamander, Amphiuma, and Batrachoseps, in which, according to Flemming, Meves (1896), McGregor, and Eisen the chromosomes are longitudinally split. But it will be impossible to determine whether this is so in the first maturation division of the ova of these Amphibia until we know accurately the mode of formation of the chromosomes themselves in the interior of the germinal vesicle.

5. The number of the chromosomes.

I have not paid a very great deal of attention to this point, but I believe the number to be fifteen in each of the two polar

divisions, and in the first polar body, though sometimes I have seemed to make sixteen, sometimes only fourteen. In the fertilization spindle I have counted about thirty chromosomes.

This disagrees with the computations of Fick, who counts eight in the polar divisions, and of Kölliker, who has given the number in the dividing nuclei of blastomeres as twelve.

B. Fertilization.

1. General outline of fertilization.

The spermatozoon may enter the egg at any point in the animal hemisphere. Its entry is accompanied by the formation at the surface of a deep pit or funnel filled with a plug, the entrance cone.

The sperm lies at the bottom of this funnel, and a clear area—the sperm-sphere—rapidly forms round the head and middle-piece.

The last named disappears; as it disappears the sperm-sphere assumes a radiate structure, the sperm-aster, and the centre of this soon becomes occupied by large vacuoles. The sperm head becomes gradually transformed into an oval sperm-nucleus which, preceded by its aster, moves into the interior of the egg and meets with the female pronucleus.

The definitive centrosome is formed in connection with the sperm nucleus, probably from it. This centrosome divides. The fertilization spindle is then formed between the two centrosomes, the male and female pronuclei breaking up independently into chromosomes in its equator.

I cannot state the time occupied by these processes with very great certainty. The female axolotl begins depositing her ova soon after midnight or early in the morning, and continues laying at short intervals throughout the early part of the day. It is necessary to watch the animal closely and remove each batch of eggs as soon as it is laid; but even so the time of laying can only be ascertained approximately.

In this way I have found that the entry of the spermatozoon and the formation of the sperm-sphere takes about two hours, the formation of the sperm-aster, the disappearance of the middle-piece, and formation of the two pronuclei about five hours. About seven hours after laying the pronuclei have met, while the definitive centrosome has made its appearance and divided into two; and about two hours later the fertilization spindle is complete. These observations were made in March, 1901.

Fick makes the whole time much shorter, but he carried on his work later in the year.

2. The entry of the spermatozoon.

I have not observed the actual entrance of the spermatozoon.

In the earliest stage in my possession the sperm—the tail of which is taken into the egg with the head—is seen lying in a clear area of cytoplasm in the midst of the yolk-granules (fig. 15). This clear area, which I will call the sperm-sphere, since it corresponds to what has been described under that name by other authors, lies at the inner end of a deep funnel-shaped depression of the surface of the egg. The superficial pigment of the egg is continued down the sides of this depression to the bottom (fig. A). The funnel itself is occupied by a plug of clear hyaline coagulum, apparently of some watery substance, which projects slightly at the mouth of the funnel, and is here surrounded by a circular groove; its outermost layer is very dense. The whole is covered continuously by the vitelline membrane. This plug is the entrance cone (wrongly termed by earlier observers the cone of attraction), formed on contact of the sperm with the ovum; it has been observed in numerous cases.

The substance of the plug is later on invaded by the surrounding pigment and yolk-granules. Its position in the egg is thus marked by a track of pigment, which may be termed here, as it has been in other cases, the "penetration" path of the sperm.

At its bottom the entrance-funnel widens out into the sperm-sphere already alluded to. This is an area of yolk-free cytoplasm possessing a finely reticular or alveolar structure—which I must leave an open question—and containing scattered about in it a few pigment granules. The spermatozoon lies in it in such a manner that the middle-piece here, as in the salamander and other Urodela, a very

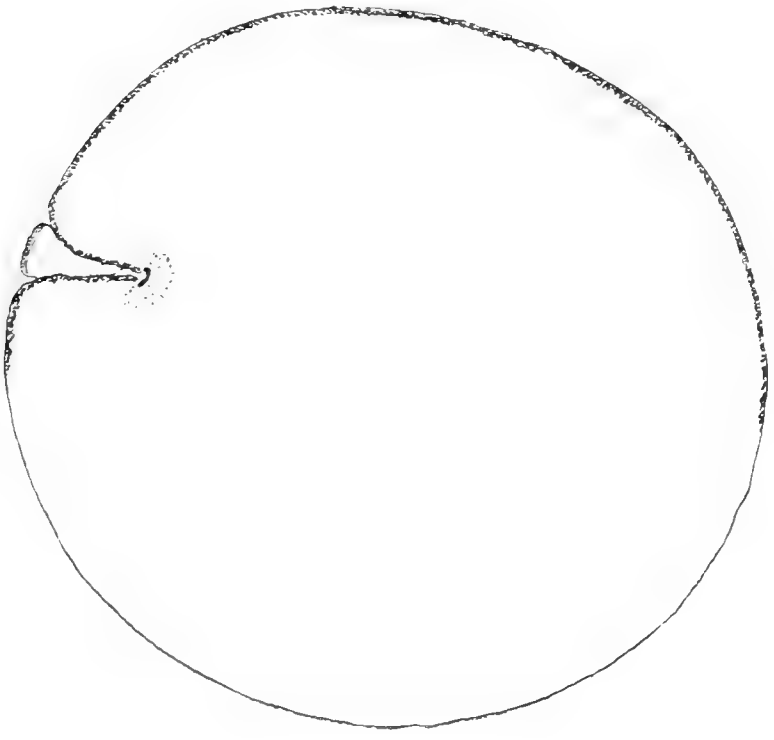

Fig. A.—Outline camera drawing of a section parallel to but not actually including the egg-axis. The section shows the entrance cone and funnel and the spermatozoon lying in a clear area at the bottom of the latter, the sperm-sphere. The superficial pigment of the animal hemisphere is represented, but the yolk-granules are omitted. The sperm-sphere is dotted.

large, easily distinguishable body, lies nearest the interior of the ovum, while the head and tail, bent on one another at this point, are both directed outwards up the entrance-funnel. It is as though the apical body of the sperm-head had on entering been caught amidst the yolk-granules, and the middle-piece then been swept onwards into the interior of

the egg. In immediate proximity to the sperm-head are a few clear vacuoles.

The structure of the axolotl-spermatozoon is well known, and closely resembles that of the salamander and newt. The head is very long, and tapers to the apex, the tail is even longer, and provided with an undulating membrane or fin. The middle-piece is embedded in the posterior end of the head, and stains less deeply than the latter with iron-hæmatoxylin, while with gentian-violet and orange, and borax-carmine and picro-indigo-carmine it takes in each case the plasma stain. This middle-piece is derived in the axolotl—as Meves (1897) has shown it to be in the salamander—from one of the two centrosomes of the spermatid.

The sperm may enter at any point in the animal hemisphere, and sometimes even a little way below the equator.

In the Axolotl polyspermy is normally of frequent occurrence, and two sperms may even enter by the same funnel. There is nothing to distinguish the accessory spermatozoa from that one which copulates with the female pronucleus. The changes they all go through are similar and practically synchronous, and centrosomes are formed—as we shall see later on—in connection with them all. There is no fact that I am aware of to indicate that this process is pathological; it must, on the contrary, be compared with the exactly similar physiological polyspermy observed by Rückert (1899) in Elasmobranchs and by Oppel and Nicolas in Reptilia. Of the ultimate fate of these accessory spermatozoa I am not in a position to say anything.

3. Changes in the spermatozoon; development of the sperm aster; disappearance of the middle-piece.

The sperm-head soon begins to shorten and thicken; at the same time a few small vacuoles make their appearance in its substance, which thus comes to have an extremely coarse reticular appearance (fig. 16). This is the first indication of the

transformation of the sperm-head into the sperm-nucleus. I believe, however, that the tapering apical extremity of the sperm-head is not used in this process, but is cast off, and degenerates in the cytoplasm. At any rate I have noticed in some of my preparations a chromatic body placed near the sperm-head, or in the sperm path, sometimes filamentous and twisted, sometimes rounded and vacuolated, which seems to be the remnant of this portion of the spermatozoon.

The sperm-head lies a little to one side of the sperm sphere, sometimes just outside the sphere between the yolk-granules. The tail makes an angle with it as before, but is completely severed from it, and there is no trace whatever of the middle-piece. Instead the centre of the sphere is occupied by a spherical vacuolated mass in which no pigment granules are found.

The sphere itself has meanwhile assumed a radial structure. Arising from the outer surface of the central vacuolated mass are numerous filamentous processes—as they appear in sections. These processes radiate in all directions, and are continued outwards for some distance between the yolk-granules beyond the limits of the sphere, disappearing finally into the general cytoplasm of the egg. They constitute the well-known sperm-aster. These filamentous rays are united to one another by frequent anastomoses, and the structure presented by the whole is that of a large number of elongated chambers, or alveoli, radially arranged; this interpretation is borne out by the appearance of a section tangential to the sphere, which is that of a polygonal meshwork, thickened at the nodes. The spaces—whether alveoli or not—between the rays and their anastomoses are filled with a faintly-staining coagulum. Pigment granules are scattered freely, but not abundantly throughout the sperm-aster, as in the stage last described, but are absent from the vacuolated central mass.

I believe, though I cannot positively assert, that this central mass originates from the dissolution of the middle-piece; I have one preparation (fig. 17) in which a small faintly staining irregular vacuolated body is found near the centre of

the sperm-aster, and separated from the sperm-head; this body, I think, may be the last remains of the structure in question, though it is possible that it is the remnant of the tail.

But whether it dissolves in this fashion, or whether it is withdrawn into the sperm nucleus—as I suppose is a not impossible view—of its actual disappearance there cannot be the shadow of a doubt. In a stage which is, to judge by the further shortening and thickening of the sperm-head and by its increased vacuolation, more advanced than that just under discussion, no sign of the middle-piece can be seen (fig. 18); the centre of the sperm-aster is occupied, as before, merely by a vacuolated mass. The tail has also now disappeared.

4. Formation of the pronuclei; appearance of the definitive centrosome.

(a) The female pronucleus.—The chromosomes left in the egg lie in a small, clear area. At first they converge by their apices (fig. 8), as in the anaphase, but presently become arranged in a tangled skein, without, however, losing their individuality. A little later still a nuclear membrane appears, surrounding the chromosomes (fig. 28, *a*.). These lie in an achromatic network; but whether this is derived from the chromosomes or not I cannot say. It certainly stains differently, but at the same time the surfaces of the chromosomes are everywhere produced into small, tooth-like processes, which lends some colour to the view that the achromatic network is in reality the result of the continued outgrowth of these.

The chromosomes become broken up into at first coarse (fig. 28, *b*. and *c*.), but ultimately very fine fragments, which are evenly distributed over the achromatic reticulum; these small granules seem to lose much of their staining capacity (fig. 28, *d*. and *e*.). It is not possible to speak very positively, but it seems as though a great deal of the chromatin had gone into solution in the nuclear sap. In any case

the persistent identity of the chromosomes cannot possibly be maintained for an instant.

The female pronucleus thus reconstituted begins to move into the interior of the egg; at the same time it enlarges considerably, and becomes irregularly lobed. It is, as a rule, closely surrounded by the yolk-granules, but a few vacuoles may be developed in its immediate proximity (fig. 28, *d.*); this, however, is not of frequent occurrence. It cannot be traced to any action of the preserving fluid. True achromatic nucleoli appear later on in its interior; these bodies stain very deeply with the plasma stains, eosin and indigo-carmine, and also very deeply with iron-hæmatoxylin. They may be slightly lobed and vacuolated (fig. 27).

(b) The male pronucleus.—Ultimately the male pronucleus has precisely the same structure as that just described for the female, but this structure is arrived at simply by a continued process of vacuolation. At no time in the transformation of the sperm-head is it possible to detect any separate chromosomes.

In the stage last described the sperm-head was in the form of an obtuse cone (fig. 18). The substance of this cone, which is highly chromatic, now becomes considerably vacuolated. The vacuoles vary in size; many of them are so close together that only a thin separating lamella is left. By a continuation of this process the nucleus comes to assume a typical reticular structure (figs. 26, *b.*; 20). The coarse, and now achromatic, reticulum is apparently derived from the remains of the lamellæ, while the chromatin is confined to the large, often irregular granules at the nodes. Gradually, however, the reticulum becomes much finer, the chromatin more minutely divided and less intense in its staining reactions, while true nucleoli make their appearance (figs. 19, 21). The male pronucleus is now exactly similar in structure to the female. Like the latter also it is at first rounded but subsequently irregularly lobed, and undergoes a marked increase of volume.

Though the above seems to be the normal series of changes which the sperm-head passes through, a slight variation of

this process sometimes occurs (figs. 23, 29, 36). The chromatin may become crowded together in the centre of the nucleus, and here form a compact, coarse, deeply staining reticulum, the surrounding intra-nuclear space being occupied by an achromatic substance which is sometimes homogeneous, sometimes reticular. The male pronucleus may be observed in this condition even in the fertilisation spindle, in which case the chromosomes seem to be formed directly from this chromatic network without the intervention of a typical resting stage.

(c) Appearance of the definitive centrosome.—In the previous stage the centre of the sperm-aster was occupied by a vacuolated mass. These vacuoles now swell up enormously and assume a radiate arrangement about the centre of the aster (figs. 19, 21, 24). The separating lamellæ between them become so extremely thin and delicate as to be almost invariably ruptured during the process of fixation or subsequent passage through the alcohols. Consequently the centre of the aster seems to be occupied by one great vacuole, the cavity of which is traversed by irregular broken strands, the remains of the thin inter-vacuolar lamellæ (fig. 30). A few pigment granules may be seen dotted along these strands, but they are much more numerous around the periphery of the large vacuole. They are also to be seen in the outer zone of the aster.

This latter has still the same structure as before, that is to say it consists of a system of radiating fibres connected by numerous anastomoses and continued outwards for some distance between the yolk-granules. As before the spaces between these fibres or lamellæ—whichever they may be—are occupied by a faintly-staining coagulum; the large central vacuole, or vacuoles, is occupied by a coagulum of precisely the same nature.

This substance would appear to be of more watery consistency than the rest of the cytoplasm. The formation of the large vacuoles is in that case to be looked on as a concentration in the centre of the sperm-aster of water withdrawn

—probably under the immediate influence of the middle-piece—from the cytoplasm of the egg. If so, this is a fact of the very highest physiological importance in the process of fertilisation. I must however defer the full discussion of it to another part of this paper.

The sperm-nucleus lies a little to one side—the outer side—of the sperm-aster; and as soon as the large vacuoles are formed projects slightly into them. These then appear as a system of clear spaces partially surrounding the inner side of the sperm-nucleus and preceding it in its progress into the interior of the ovum to meet the female pronucleus. The path, generally termed the "copulation" path, which the sperm-nucleus now pursues is not as a rule in the same straight line as its earlier "penetration" path, but makes an angle with it.

It is during this stage, when the sperm-nucleus is already coarsely reticular, that the definitive centrosome appears (figs. 19—21). This is a large rounded body, composed of a granular substance staining faintly with carmine, and not very deeply with iron-hæmatoxylin. Occasionally one or more intensely-staining granules may be discerned in its interior. Its diameter is about one-quarter or one-third that of the sperm-nucleus. It is always surrounded by a cloud of pigment which may be so dense as to entirely obscure the centrosome within (fig. 23); this can, however, easily be demonstrated after depigmentation with the fumes of nitric acid (fig. 22). It lies in front of the sperm-nucleus, between it and the system of vacuoles. When the sperm-nucleus comes to project into the vacuoles the centrosome occupies approximately the centre of the system.

This body is also found in connection with the accessory sperm-nuclei, where it has exactly the same character and behaves in precisely the same manner (figs. 19, 22, 23, 24).

The centrosome very soon divides in a direction which is at right angles to the "sperm" path (fig. 22). Preliminary to division it becomes elongated and constricted (figs. 20, 21). The halves may be at first connected by fibrillæ. In one case

I have observed the two halves united by two curved rods, the whole having the appearance of an oval ring (fig. 27).

The diverging halves move apart till they are separated by a distance a little greater than the longer diameter of the nucleus. The division usually occurs before the pronuclei have met, but it may be deferred (fig. 27).

With regard to the mode of origin of this centrosome I do not wish to speak too positively. It may be argued, in view of the known persistence of this organ from one cell-generation to the next in cases of ordinary division, that the centrosome must arise here also from the middle-piece, which, as we know, is itself merely the enlarged centrosome of the spermatid. In this case we should have to suppose that the middle-piece, after being dissolved in an early stage became reprecipitated in a later. The solution and reprecipitation of a nuclein is of course no very extraordinary process; it occurs quite normally in the nucleus in the disappearance and re-formation of the chromosomes.

Now, however much may be said for such an hypothesis from a purely theoretical and comparative point of view, it is hardly supported in the case of the axolotl by any positive evidence at all, and is, as I believe, directly negatived by the evidence which I am able to bring forward in favour of a totally different origin of the centrosome, namely, from the sperm-nucleus itself.

I have observed in many cases that the membrane of the sperm-nucleus cannot be detected, or is at least very much weakened on the side turned towards the centrosome (figs. 22, 23), and in some preparations the centrosome is so closely apposed to this side of the nucleus that it appears to be actually emerging from it (figs. 24, 25). The dense cloud of pigment which, as we have seen, obscures the centrosome, appears to come into existence simultaneously, for deeply pigmented processes are observed passing inwards from the centrosome into the interior of the nucleus. To judge by this evidence, then, centrosome and pigment are both formed not

merely in connection with but through the active agency of the sperm-nucleus.

It cannot indeed be said that the centrosome is, literally, of intra-nuclear origin, for no formed body at all like it is ever observable in the interior of the sperm-nucleus. What does however seem to me probable is this, that this body is produced through the precipitation of albumins or globulins present in the cytoplasm by nucleic acid or nucleins emerging from the nucleus, a view which coincides with that advanced by Fischer of the formation of the centrosome in general.

The origin of the pigment, on the other hand, is a matter about which I hardly care to advance any conjectures; but I think it is certain that it is too abundant to allow us to suppose that it has been dragged in by the spermatozoon on its entrance into the egg; besides it is absent in the previous stages.

I cannot conclude this paragraph without alluding to some preparations I have which may be considered to favour the reprecipitation hypothesis mentioned first. In these a dense (fig. 26, *a*.) granular mass, undeniably like a centrosome, is found in company with a sperm-nucleus (fig. 26, *b*.), which is in an earlier stage of development than that in which the centrosome usually first makes its appearance; further, the nuclear membrane is quite intact in these preparations. Against this interpretation I must urge that the middle-piece is certainly absent at an earlier stage still, that nucleic acid may diffuse through without actually bursting the nuclear membrane, and that there is no reason why the production of the centrosome by the other method should not have taken place precociously.

5. Union of the pronuclei. The fertilisation spindle.

Preceded by its centrosome, sphere, and aster, the sperm-nucleus makes its way into the interior of the egg. The female pronucleus has meanwhile been moving away from its position at the animal pole, and sooner or later the two

pronuclei meet. Although eventually the fertilisation spindle will intersect the egg-axis, the separate "copulation" paths of the pronuclei frequently converge to a point which is not actually in this axis, and may be some distance away from it; in other cases, however, the sperm-nucleus reaches the axis before the female pronucleus has joined it. In this latter case "penetration" path, "copulation" path, and egg-axis all lie in one plane, which, since the centrosome divides at right angles to it, is the plane of the first furrow. This may then be said to be determined by the point of entry of the spermatozoon. When the point in which the pronuclei meet is ex-axial, the plane of the first furrow may possibly be determined by the "copulation" path alone, as Roux has shown to be the case in the frog.

This variability in the position in which the pronuclei first meet is obviously partly due to the variability of the point at which the spermatozoon enters the egg, and consequently of its "penetration" and "copulation" paths; but also partly to variations in the path pursued by the female pronucleus, which does not necessarily descend vertically from the animal pole towards the centre of the egg, but may diverge from the egg-axis (figs. B. and C.).

A further result of this is that the female pronucleus may come in contact with the sperm sphere at any point on its inner and upper surface.

The end is, however, always the same; the female pronucleus enters the vacuolated substance of the sphere, and comes to lie close to the sperm-nucleus, with the centrosome or diverging centrosomes between the two (fig. 29), the line joining the two pronuclei intersecting that between the two centrosomes at right angles. The large vacuoles of the sperm sphere are thus divided into two sets, one adjacent to each centrosome (fig. 31). These two sets of vacuoles usually appear in preparations each as a single large vacuole; this appearance is artificial and due to the breaking down of the thin separating lamellæ.

Although it seems clear that here, as in many other cases,

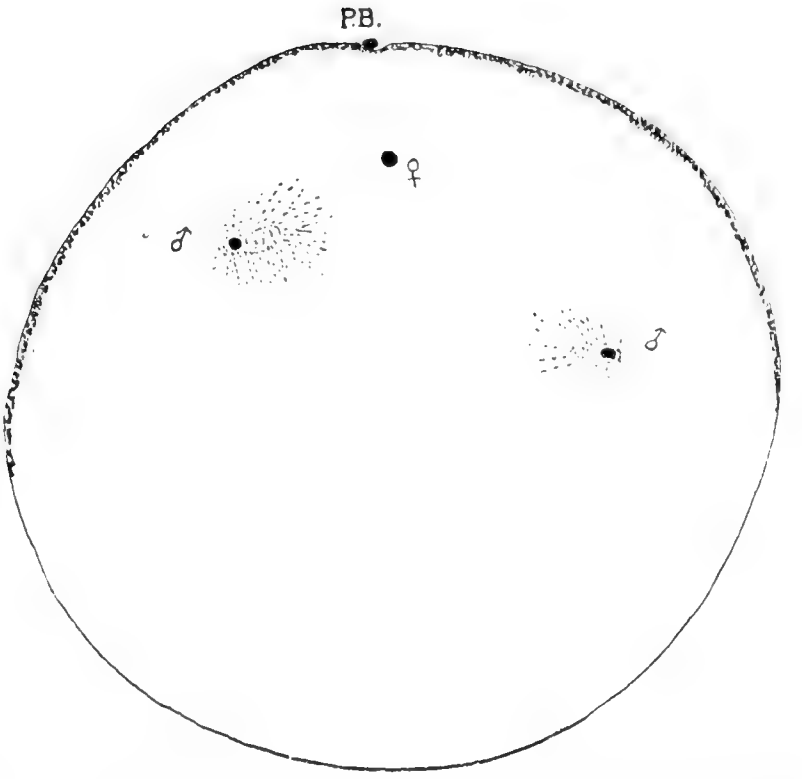

Fig. B.—Meridional section showing female pronucleus in the egg-axis, and two sperm-nuclei with their asters. A the animal pole is the second polar body. Camera drawing.

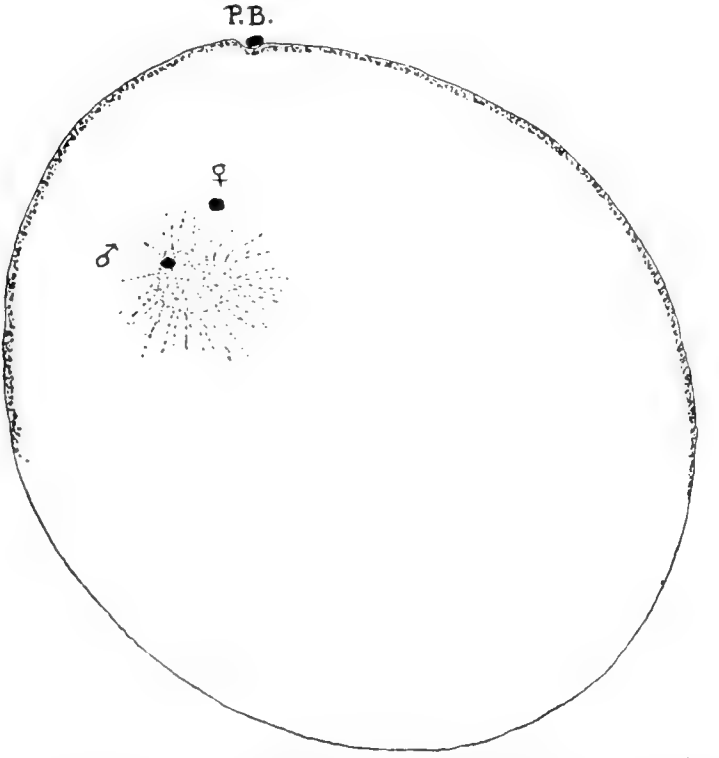

Fig. C.—Outline camera drawing of a meridional section of an egg, showing female pronucleus in an ex-axial position and sperm-nucleus with aster. The polar body is a little to one side of the animal pole.

the movements of the two pronuclei are influenced by one another, I am unable to offer any suggestion as to what the nature of that influence may be.

For a time the sphere which encloses the two pronuclei and centrosomes retains its original form, but soon it begins to elongate in the direction of the (future) spindle axis (figs. 31, 32), that is of the line joining the two centrosomes. Simultaneously the external radiations separate into two distinct terminal or polar groups, each of which centres in a centrosome; the middle or equatorial region being now devoid of radiations, and occupied merely by rounded vacuoles (figs. 32, 33).

The whole structure then moves into its definitive position in the egg-axis if it has not already reached it. This position is such that the pronuclei and centrosomes all lie in one plane which cuts the egg-axis at right angles at the distance of about one quarter of a diameter from the animal pole, the egg-axis passing midway between the two pronuclei and between the two centrosomes. Fertilisation spindles are, however, occasionally observed in an ex-axial position. The result of this is, of course, that the first furrow is not accurately meridional, a fact of frequent occurrence.

The formation of the fertilisation spindle now begins. The first sign of this is the outgrowth of fine, nearly parallel fibres from the centrosomes towards the pronuclei (figs. 31, 32). Here, again, there is reason to believe that these spindle fibres are in reality the optical sections of inter-alveolar lamellæ; each has a conical base at its point of attachment to the centrosome, and also at its opposite end where it touches the nuclear membrane. The inter-fibrillar spaces have, therefore, the appearance of extremely elongated elipses. It is of interest to observe that such spindle fibres may grow out from the centrosome towards an accessory sperm-nucleus (fig. 19).

The centrosomes remain for a time united by a narrow, deeply pigmented cord (fig. 32); this sooner or later breaks, the centrosomes becoming pear-shaped (fig. 31), but soon assuming the spherical form.

With the formation and elongation of the spindle-fibres the centrosomes move further apart; at the same time they begin to enlarge, and continue to do so until they have attained a very considerable size (fig. 33). Pari passu with this enlargement the vacuoles—the vacuoles of the original sperm-sphere—gradually disappear. I believe that the two processes are intimately related, that, in fact, the centrosomes enlarge at the expense of these vacuoles, and that their growth consists essentially in an imbibition by them of the watery substance concentrated at an earlier period in the centre of the sperm-sphere.

This growth of the centrosomes is accompanied by the formation not only of the spindle fibres, but also of the polar asters. Under this heading are comprised all those radiations which pass outwards from the centrosomes, with the exception of those—the spindle fibres proper—which pass to the two pronuclei.

The outer ends of these astral radiations are distinguishable from the first from the spindle-fibres by their coarser structure; the fibres—or lamellæ—are stouter, the inter-fibrillar spaces—or alveoli—much wider, and seem to be identical with the earlier radiations of the sperm-sphere, separated, as we have seen, by the elongation of the latter into two distinct halves, centring each in a centrosome. The pigment which surrounded the sperm sphere is found thickly scattered about these outer rays (figs. 31—34).

The inner ends of the astral rays on the other hand, though perfectly continuous with the outer, differ greatly from them in their appearance and in the mode of their formation. In the fineness of their structure they resemble the spindle-fibres, and they occupy the space previously taken up by the large terminal vacuoles (figs. 33, 34). They may, and indeed must, I believe, be regarded as outgrowths of the centrosomes, developed at the expense of the contents of the vacuoles which they replace. The exact nature of such an outgrowth I shall have occasion to discuss later on; but I may say here that in describing the process by this term I do not mean to imply

that they consist entirely of centrosomal substance. On the contrary, I suspect that we have here to do with the precipitation of the proteids of the cytoplasm by the dissolved substance of the centrosome, in which case these outgrowths owe their origin as much to the former as to the latter.

The further metamorphosis of the centrosomes and asters is as follows:—

As stated above, the inner portion of the aster consists of thin, closely set rays in immediate contact with the centrosome. This radiate structure persists for some time, the constituent rays becoming even finer and more closely set (fig. 34). Later, however, in the fully formed spindle (figs. 38—41) the radiate arrangement is lost, and the inner portion of the aster becomes a sphere with an exceedingly fine reticular or alveolar structure. From the surface of this centrosphere start the outer astral rays; in its centre is placed the centrosome.

This body has also undergone important modifications. In the earliest stage of the fertilisation spindle the centrosomes are small, round, sometimes axially compressed bodies (figs. 31, 32); they are not coloured deeply with iron-hæmatoxylin, but may contain a larger or smaller number of granules which do stain intensely with that dye. They then, as we have seen, enlarge very considerably (fig. 33), while the fibres of the spindle on the one hand, the inner astral rays on the other grow out from them (fig. 34). When the metamorphosis of the inner portion of the aster so formed is completed the centrosome is once more small (figs. 38—41). It is not easy to see in material preserved with corrosive and acetic (figs. 38, 39), having a reticular structure distinguishable only with difficulty from the fine reticulum of the centrosphere itself. With chromic and acetic (figs. 40, 41), however, the centrosome stands out from the substance of the centrosphere as a small, compact, homogeneous body, slightly lobed, and containing a deeply staining particle, the centriole; occasionally the centriole (fig. 40), and sometimes the whole centrosome (fig. 35, *a*.) is seen to have divided. In this case the daughter

centrosomes are flattened against one another; the direction of their division is at right angles to the axis of the spindle. This division takes place as a rule during the anaphase, but I have found the centrosome doubled at an earlier stage.

The cycle of changes which this cell-organ passes through would then appear to be as follows:—At first a small body, the centrosome begins to swell by absorption of the watery contents of the adjacent vacuoles; then spindle fibres and astral rays begin to grow out at its expense in turn; finally, while the large centrosphere is being formed by the reticular degeneration of these rays the centrosome once more returns to its original volume and divides. If we choose, with Boveri, to look on the centrosphere as simply an enlarged centrosome—and I think that, with certain reservations, there is much to be said for this view—then we shall regard the small corpuscle found in its centre as a "reduced" centrosome in his sense, as coming into being by a condensation of the central portion of the larger body.

Though I have not made any extended observations on the behaviour of the centrosomes during segmentation, I may, perhaps, be allowed to give an account here of what little I have been able to make out.

In the telophase of the first division two small centrosomes may be found on the polar side of the nucleus (fig. 35); they are usually extremely hard to detect, mainly, I fancy, because they lie in a depression of the nuclear membrane. The centrosphere has, as such, totally disappeared, and with it the astral rays. Its place is occupied by a large highly vacuolated area surrounding the nucleus, and resembling exactly the system of vacuoles formed in connection with the sperm-nucleus.

In the metaphase of the dividing nuclei of blastomeres a large centrosphere is present at each spindle pole, and in the centre of this is a reticular centrosome (I have at my disposal only material preserved with aceto-corrosive) which can barely be distinguished from the surrounding reticulum. These facts seem to me to indicate that the centrosomes of the

blastomeres go through precisely the same cycle of changes as that which I have described above for the cleavage centrosomes, and that this body, when introduced into or formed in the ovum, becomes a permanent organ of the embryonic cells.

Before leaving the aster I have to describe certain changes that take place in its peripheral region.

We have seen that the centrosphere is surrounded by coarse radiations which pass out between the yoke-granules into the general cytoplasms, and appear to be identical with one half of the radiations of the sperm-aster. These radiations do not at first extend into the equatorial region of the spindle, which is occupied only by a mass of round vacuoles (figs. 32, 33); but in the fully-formed spindle a complete mantle of radiations is found wrapping round the spindle proper and extending as far as the equator (figs. 38, 41). Here the radiations meet without, as far as I can see, ever intercrossing with those derived from the opposite pole; on the contrary the two sets of rays seem to diverge outwards and to lie parallel to one another, one on each side of the equatorial plane. The rays become closely crowded together by the expansion of the nuclear spindle (figs. 38, 40, 41), and are thickly beset with pigment granules.

These equatorial astral rays thus appear to be a completely new formation, replacing the round vacuoles of an earlier period; but whether they are in reality outgrowths of the previous rays—and in this case we might have to attribute their formation ultimately to the activity of the centrosome—or whether they arise merely by the compression of the round vacuoles, is not easy to determine. The persistence of the pigment granules leads me to incline to the latter view; for I have noticed that in the case of new formations, for example in the formation of the vacuoles of the sperm-sphere, the pigment granules are swept aside. On the other view we should have to suppose that the pigment in question was pushed outwards from the centrosome by the continued growth of the rays, and this is favoured by the fact that the dense pigment which surrounded the centrosome at its first

appearance is certainly not found, except for a few sparse granules, about the fully formed centrospheres. Some of this original pigment, that between the pronuclei, seems simply to disappear in situ, but the remainder is probably carried to the periphery.

We may now return to the consideration of the spindle.

At present we have only described that portion which lies extra-nuclear—between the centrosome and the pronuclei, and arises by outgrowth from the former. These polar portions increase considerably in length before the equatorial part is formed. The extreme polar ends of the fibres become merged in the centrospheres.

The equatorial portion is most distinctly intra-nuclear in origin. The two pronuclei, greatly increased in volume and elongated in the direction of the spindle axis, are closely applied to one another. In a stage when the chromosomes are being formed the nuclear membrane appears indented at the ends, apparently by the growth of the extra-nuclear fibres. Soon openings appear in the membrane (fig. 38), and through these the extra-nuclear fibres and inter-fibrillar spaces become continuous with a similar set of fibres and spaces, each with each, which are formed inside the nucleus by a re-arrangement of the achromatic reticulum. In other words, the threads of this reticulum, previously irregularly distributed, became now parallel to the axis of the spindle, and continuous through the openings in the membrane with the fibres outside.

This is, I think, a fair account of the appearances of sections; whether it is a true description of what actually occurs is another matter. I have indicated briefly above that the inner rays of the aster and the extra-nuclear spindle fibres may possibly be regarded as produced by the precipitation of the albumins of the cell by a substance derived from the centrosome; in the same way these intra-nuclear fibres may be regarded as produced by an extension of the process, that is to say by the precipitation through the same agent of the albumins of the pronuclei themselves. I shall discuss the point in greater detail further on.

With the completion of this process and the total disappearance of the nuclear membranes, which seem to be used in the formation of the fibres, the spindle may be said to be fully established. It consists now of undulating fibres passing continuously from one pole to the other, and frequently united by anastomoses (fig. 39). Transverse sections show a polygonal meshwork thickened at the nodes; we have as good reason here as in other cases for regarding the fibres as the optical sections of inter-alveolar lamellæ. The spindle increases in diameter as well as in length.

Very considerable changes have been meanwhile taking place in the pronuclei also.

In the early fertilization spindle they are round, somewhat irregular bodies, much increased in volume since their first formation. They possess a fine achromatic reticulum, chromatin in a state of minute subdivision, and true nucleoli or plasmosomes (figs. 31, 32). In this condition they remain during the early stages, except that they become enlarged and lengthened in the direction of the spindle axis (fig. 32), but when the latter is beginning to elongate the chromatin granules increase both in size and number (fig. 33). The total quantity of chromatin in the nucleus seems therefore to be greater than before, as though it had been reprecipitated from solution.

Of the first steps in the production of the chromosomes I can say very little (fig. 36). In the earliest stage which I have irregular moniliform chromatic threads are scattered through the nucleus; their length is variable, and they appear to be in process of formation by the linear aggregation of granules. In this stage the nucleoli are still to be seen, but later they disappear. The chromosomes certainly do not arise directly from them.

The chromosomes appear separately in each pronucleus, while the nuclear membranes are still intact (figs. 34, 38). Each chromosome is a twisted rod of uniform thickness, showing very little, if any, traces of the earlier moniliform structure. The chromosomes lie scattered throughout the

pronuclei quite independently of the achromatic reticulum. This has now assumed a much coarser arrangement than before; there are very obvious granular thickenings at the nodes.

With the disappearance of the nuclear membrane and the completion of the spindle, the chromosomes are thrown on, or rather in, the equator of the latter in two distinct groups, derived from the two pronuclei, as may readily be seen in transverse sections (fig. 37). The Axolotl is therefore one of those very numerous forms in which no "segmentation nucleus" is formed, but the maternal and paternal chromosomes preserve their individuality in the fertilisation spindle.

The chromosomes at first project to one side and the other of the equatorial plane (fig. 39), but soon lie wholly in it. They then split longitudinally (fig. 40). Further they are not merely placed on the periphery of the spindle, but are scattered throughout it.

It is at this stage that certain bundles of fibres first become distinguishable from the general fibres of the spindle (fig. 40). These bundles—the "Zugfasern" of cytologists—are attached by their equatorial ends to the chromosomes; at their polar ends the constituent fibres separate and become lost in the general fibrillo-reticulum. The bundles from the opposite poles of the spindle are arranged in pairs, a pair for every pair of chromosomes; the two bundles of a pair are attached exactly opposite to one another one to each chromosome, at or near one end of the latter.

In the anaphase the chromosomes diverge by these ends (fig. 41), which become hooked when the point of attachment is not actually terminal. No trace of the bundles can be seen between the chromosomes, and the whole appearance most decidedly lends support to the view that the bundles are the actual agents which pull the chromosomes apart, the latter being quite passive during the process. At the same time though the bundles shorten they never, as far as I have seen, thicken; we have, therefore, here no evidence at all that the "Zugfasern" contract like muscle-fibres, and that

their behaviour can be explained simply by comparison with these.

After the separation of the chromosomes the general spindle-fibres remain behind. An achromatic equatorial plate (the cell plate) is now clearly visible (fig. 41), though indications of it may indeed be seen in the metaphase (fig. 40). This plate consists of a thickening and union of the fibres in the equatorial plane. Axially, the spindle-fibres are perpendicular to this plate; outside the axis they make an angle with it, more peripherally still they curve outwards and lie parallel with it. Where the fibres meet the plate they are thickened. It looks as though two opposing sets of alveoli had here met and fused. What relation, if any, this equatorial plate bears to the subsequent cytoplasmic division I cannot say.

In the telophase the nucleus becomes once more completely reticular, and the plasmosomes reappear. Its polar surface is deeply indented (fig. 35). The division of the centrosome, the degeneration of the centrosphere, the formation of large vacuoles round the nucleus have already been described.

6. Remarks on the work of Fick and Michaelis.

The foregoing account differs seriously from that given by Fick in one important particular, the origin of the definitive centrosome.

After describing the formation of the sperm-aster about the middle-piece, and showing that the latter becomes separated from the sperm-head, swells up and loses the distinctness of its outline (in all of which I am able to agree with him entirely), Fick proceeds as follows: "Die Attraktions-sphäre zieht ihre Strahlen ein, ballt sich zusammen zu einer intensiv roth-gefärbten Kugel oder zu einem Unregelmässig gestalteten abgerundet eckigen Klumpen, ganz ähnlich wie die von Boveri bei Ascaris abgebildeten Archoplasmaklumpen."

This, preceding the sperm, divides to form the centrosomes (though he does not apply this term to them) of the fertilisa-

tion spindle. The cleavage centrosomes, therefore, are derived from the middle-piece which is, as Fick surmised and as we now know, the enlarged centrosome of the spermatid.

As I have tried to show, such a view is untenable; for not only is there a stage in which the middle-piece has clearly disappeared, but also we have direct evidence for the formation of the definitive centrosome de novo from the sperm-nucleus.

The point is one of considerable theoretical importance. Up till now the Axolotl has been the only form in which the persistence of the centrosome from the spermatid to the fertilisation spindle could be positively asserted; for though on the one hand the origin of the middle-piece from the previous centrosome has been traced in many cases, while on the other there are numerous observations of the formation of the fertilisation spindle by division of the sperm-aster, both processes had been seen in no animal but this.

In several other respects I have been able to go into greater detail than Fick; the polar spindles, the structure of the sperm-aster, and notably the formation of the fertilisation spindle. Fick's description of the last is indeed very deficient.

On the other hand he has described the mode of entry of the spermatozoon and the entrance-cone and funnel. The entrance-cone is, according to him, an aggregation of "Eiplasma," and is produced by something in the nature of a ferment provided by the spermatozoon. It has a dense, radially striated border.

More recently Michaelis has published a short paper on the fertilisation of a closely-allied form—the newt.

His observations on the fate of the middle-piece agree closely with my own. Radiations appear at an early stage, but "dass dei genannten Strahlungen in irgend einem Zusammenhang mit der späteren Attraktions-sphäre stünden ist kaum anzunehmen." Later there comes a stage in which "vom Mittelstuck ist nichts mehr zu sehen."

He has failed to find any cleavage centrosome, though it

can hardly be doubted from the work of van der Stricht (1892) and Braus that such exists in segmentation stages.

On another small point I must disagree with Michaelis. He says there is a segmentation nucleus. I find, on the contrary, in some preparations of fertilisation spindles of Triton which I have, that there are two distinct sets of chromosomes. At the same time we ought to bear in mind Boveri's (1890) assertion that in one and the same species of Echinus there is a variation in this respect.

III. Historical and Critical.

A. Maturation.

1. Structure of the polar spindles.

In a series of elaborate and valuable memoirs Carnoy and Le Brun have described the formation of the polar spindles and bodies in both Anurous and Urodelous Amphibia. Their observations are very complete and detailed, but do not differ in any other important respect from my own.

The first polar spindle is of intra-nuclear origin, arising from a special portion of the germinal vesicle—the "plage fusoriale." Both first and second polar spindles are described and figured with inner or bi-polar and outer or mantle fibres. In many cases, especially in the early stages of their formation, the poles are surrounded by astral radiations. The authors fail to find any centrosome beyond the somewhat indefinite body into which the spindle fibres converge. But that Carnoy regards this body, as I do also, as a physiological centre, seems to follow from his remark that some substance comes from the nucleus—"qui agit sur le réseau et y produit les mêmes irradiations que si ces substances provenaient d'un centrosome véritable."

In the Trout, according to Behrens, the maturation spindles have this same structure. In Amphioxus (Sobotta, 1897) only the second polar spindle is provided with mantle fibres, while in the Mouse (Sobotta, 1895) these fibres are absent in both the first and second.

In Invertebrates it is the very general rule for the asters and centrosomes of the polar spindles to be well developed (Platyhelmia [Francotte, van der Stricht (1898), von Klinckowstrom, Gardiner, Henneguy, Goldschmidt, Halkin], Nemertines [Coe, von Kostanecki (1902)], Mollusca [Lillie, von Kostanecki (1896), Boveri (1890), Mark, Linville, Griffin, Garnault], Chætopoda [Foot, Vejdovský, Korschelt, Griffin], Arthropoda [Ishikawa], Echinoderms [Matthews], Ascidia [Castle]); but centrosomes are stated to be absent in Ascaris by Boveri (1887), though this is denied by Carnoy and others; in Sagitta by the same observer (1890), and by Brauer (1892) in Branchipus.

Considering the wide-spread occurrence of the centrosome as an active cell-organ I believe that the ill-defined body which is undoubtedly present in these cases at the spindle pole may be looked on as a physiological centre, even though it contains no corpuscle which will react to the iron-hæmatoxylin stain; and considering what we now know of the growth and metamorphosis of the centrosome it ought not to surprise us that this body should in certain cases not merely cast off the peripheral portion of its substance, as it admittedly does, but wholly disappear into the aster to which it gives rise. I shall have to recur to this point later on.

Many authors besides Carnoy have attributed to the first polar spindle an intra-nuclear origin, either in whole or in part.

In Ascaris (Boveri [1887]), in Branchipus (Brauer [1892]), and in Ophryotrocha (Korschelt) the germinal vesicle becomes directly transformed into the spindle.

In other cases the nuclear membrane disappears under the influence of the astral rays, and the equatorial portion of the spindle arises in the interior of the nucleus (Polyclada [Francotte and van der Stricht (1898)], Cerebratulus [Coe] and others). Such a double—extra- and intra-nuclear—origin of the fibres also occurs in the fertilisation spindle. I have described this above for the Axolotl; it has also been observed in Polyclada, Cerebratulus, Thalassema, Ophryo-

trocha, Rhynchelmis, and Toxopneustes; and in Ascaris, according to von Erlanger, but not Boveri (1888).

The slight temporary depression at the surface of the egg over the polar spindle which I have noticed in the Axolotl has been seen by others also (Francotte, Griffin, von Kostanecki [1896], Linville).

2. Reduction of the chromosomes.

It is no part of my programme to enter at any length into this vexing and perhaps fruitless controversy.

As far as the Amphibian ovum is concerned, however, it is clear from the careful work of Carnoy that in the second maturation division the chromosomes are split longitudinally. What happens to them in the first polar spindle is more difficult to determine, as this depends, as I have pointed out above, very largely on the view we take of the manner of their formation in the first instance.

On this matter there are two conflicting opinions. According to the observations of Born on Triton—and Rückert (1892) has made similar statements for the Elasmobranchs—the chromosomes persist in the nucleus throughout the whole period of growth of the oocyte, although they cease to be chromatic; at the time of maturation the chromosomes of the first polar spindle are formed from them, quite independently of the numerous chromatic nucleoli which are present in the germinal vesicle and cast out into the cytoplasm when the nuclear membrane disappears. This view has been adopted by Miss King in her researches on the maturation of the toad's egg.

The other view is that advocated originally by Schulze and later by Carnoy and Fick (1899). According to Carnoy the chromosomes of the young oocyte are disintegrated. The chromatin passes into a state of solution and is continually being reprecipitated—as nucleoli—and redisintegrated and dissolved during the long period of growth of the oocyte. During this period the yolk-granules are deposited in the

cytoplasm. The formation of the yolk seems indeed to be intimately related to the solution of the chromatin, for some of this dissolved substance passes through the nuclear membrane and contributes to the nuclein which can be demonstrated in the yolk. It is during these processes of disintegration that the figures are produced which have been mistaken by Born and Rückert for chromosomes.

At the time of maturation the nuclear membrane disappears and some of the chromatic nucleoli are used in the production of the chromosomes in a very complicated fashion. According to Carnoy the resulting division is longitudinal, but I think it must be conceded that when, as here, there is no spireme stage, when the chromosomes are formed from round nucleoli, it is almost idle to attempt to distinguish between a longitudinal and a transverse division.

It will be convenient to discuss briefly at this point two questions which are raised by the subsequent behaviour of the pronuclei. The first relates to the theory of the persistent individuality of the chromosomes.

I have found no evidence in my preparations and very little in the literature in support of this assumption. Carnoy's account of the history of the chromatin is, of course, diametrically opposed to it.

The second question is the formation of a segmentation nucleus. This has been seen in Elasmobranchs (Rückert [1891, 1899]), the Trout (Behrens), Petromyzon, Amphioxus (Sobotta [1897]), Cerebratulus (Coe), Prosthiostomum, Thalassema, Toxopneustes, and Ciona (Castle, but not Boveri [1890]).

In other cases the chromosomes arise from the two pronuclei in two separate groups.

The distinction, however, seems to be worth little; Boveri (1890) has shown that in Echinus microtuberculatus both modes may occur, Michaelis has described one mode, myself the other in Triton, and Sobotta (1895) found in the Mouse one isolated case of a segmentation nucleus.

B. Fertilisation.

In the act of fertilisation two distinct processes are involved. The first is the union of two cells, the bearers of those hereditary characters which reappear in the offspring sprung from the union. The second is the restoration to the germ-cells of their lost power of reproduction by division. That this is true of the egg-cell is obvious, and is proved to be so in the case of the spermatozoon, or at least of its nucleus, by the experimental production of a larva from the fertilisation of an enucleated fragment of an egg.

It is with the second only of these two processes that I am here concerned. In it a stimulus is conveyed to the ovum by the spermatozoon, under the influence of which it divides and gives rise to a new multicellular organism.

All the recent work on the subject has been devoted to the discovery of the mechanism by which this is effected. On the one hand we see in the purely descriptive treatises of the past few years, a constant effort to ascertain the part played by the sperm-centrosome in the process, in short to test the hypothesis, first put forward by Boveri, that the sperm-centrosome is the active agent in the act of fertilisation. Nor has experimental proof of the theory been lacking. Boveri himself showed that a sperm-centrosome will divide in an enucleated blastomere, which, as Ziegler was able to demonstrate, may itself divide too. On the other hand the work on artificial parthenogenesis initiated by Loeb has suggested that the stimulus so given to the egg may be described in physical or chemical terms.

It is this theory of Boveri's that I propose in particular to discuss. In doing so it will be convenient to consider separately the phenomena accompanying the entrance of the spermatozoon, and the formation of the cleavage—or fertilisation—spindle.

1. The entry of the spermatozoon.

The time at which the spermatozoon enters the ovum varies in different forms.

In Ascaris the entrance takes places while the nucleus of the primary oocyte is yet intact; the same is true of Nereis (Wilson), Myzostoma (Wheeler), and some others. In others again the sperm enters during some stage of the first polar spindle (Ophryotrocha [Korschelt], Chætopterus [Mead], Physa [von Kostanecki], Sagitta [Boveri], and many more); or again the entrance may be deferred until the first polar body has been extruded and the second polar spindle formed, as, for example, in Amphioxus (Sobotta), Petromyzon (Böhm), the Trout (Behrens), the Newt (Michaelis), the Mouse (Sobotta), and the Axolotl, or even until the second polar body also has been given off (Toxopneustes [Wilson, 1895], Echinus [Boveri], Tiara [Boveri, 1890]).

It is an interesting speculation whether in the cases first mentioned the formation of both polar bodies, or of the second only, is dependent on the entrance of the spermatozoon. Fick has surmised that this is so in the Axolotl, and Mead in Chætopterus; while Boveri makes the same suggestion for the species of Sagitta investigated by him, though he quotes an observation of Fol's on another species that the polar bodies will form in any case, though much more slowly in an unfertilised egg. With this may be compared Hill's statement that in Phallusia the formation of the polar bodies is independent of fecundation.

That an immediate change is wrought in the cytoplasm of the egg by the entrance of the spermatozoon is proved by an interesting experiment of Ziegler's, in which the egg is divided into two pieces, one containing the egg nucleus, the other the sperm and centrosome. The latter segments normally; the former makes amœboid movements and attempts at division, while its nucleus repeatedly passes through the initial stages of division but is each time reconstituted.

The place of entrance of the spermatozoon often varies in the same species; this can naturally only occur when there is no micropyle. We have seen such a variation in the Axolotl; it is also found in Amphioxus (Sobotta), Diaptomus (Ishikawa),

Pterotrachea (Boveri), Cerebratulus (Coe), Physa (von Kostanecki).

The tail of the spermatozoon may be left outside (Toxopneustes [Wilson], the Mouse [Sobotta]); but in the great majority of cases, of which the Axolotl is one, is taken into the egg (Polyclada [Francotte and van der Stricht], Amphioxus [van der Stricht], Polystomum [Halkin and Goldschmidt]). It always degenerates.

An entrance funnel and cone similar to those observed in the Axolotl have been seen in Myzostoma (Wheeler), Ophryotrocha (Korschelt), Toxopneustes (Wilson), Insects (Henking), Petromyzon (Böhm and Herfort), Unio (Lillie), Allolobophora (Foot), and Rhynchelmis (Vejdovský).

The most accurate description of the formation of this structure is that given by the author last named.

According to Vejdovský there are outside the yolk two layers, an external alveolar sheet, and a granular plasma zone. As soon as the first has been pierced by the head of the sperm, the second is depressed to form the entrance pit or funnel. While this funnel becomes filled by a granular mass, derived by Vejdovský from the ground-substance of the cytoplasm, the alveolar sheet covering it is much thickened, protrudes outwards and exhibits a radial striation. This corresponds exactly to the outer dense zone seen by Fick and myself in the Axolotl. Later the entrance cone breaks up and disappears.

A very similar entrance cone is described by Miss Foot in Allolobophora, and by Lillie in Unio; it is termed by the latter merely the sperm-path.

Miss Foot and Vejdovský have suggested that the acrosome is the organ which is actively concerned in the production of this structure. It is interesting to notice that according to Meves, the acrosome of the Salamander and Guinea-pig, and according to von Lenhossék that of the Rat, arises from the sphere of the spermatid.

The "Pol-plasma" observed by both Böhm and Herfort in Petromyzon is essentially a cone of entrance.

We have seen that in the Axolotl soon after the entrance of the spermatozoon, the head and middle-piece become surrounded by a clear area devoid of yolk-granules. Such a sperm-sphere is of wide-spread if not of universal occurrence. Without stopping now to inquire into its physical significance I may quote a few of the cases in which it has been seen.

It has been described by Griffin in Thalassema, by Lillie in Unio, by Castle in Ciona, by Gardiner in Polychœrus, by Henking in Insects, by both Coe and von Kostanecki in Cerebratulus, and by Vejdovský in Rhynchelmis.

Both Coe and von Kostanecki express the opinion that the yolk-granules are driven away by the formation of the sphere, while Castle and Vejdovský hazard the conjecture that the sphere grows by the addition of material brought to it by streams of protoplasm moving along the surrounding astral rays.

In the Axolotl the sperm-sphere becomes subsequently vacuolated. Such vacuoles have been observed by Vejdovský in Rhynchelmis, by Herfort in Petromyzon, and by Oppel and Nicolas in Reptilia.

2. The centrosome in fertilisation.

(a) The centrosome as an organ of the cell.

(i) Intra-nuclear origin of the centrosome.

In the Axolotl the definitive centrosome is derived from the male pronucleus, through what I must regard as a precipitation of the egg-cytoplasm by the nucleins of the sperm. Although no such mode of formation of the cleavage centrosome has up to the present been described by any author (except by Carnoy in Ascaris), there are yet several instances on record of the intra-nuclear origin of this body in germ-cells.

The case which stands nearest to my own observation, is that of Styelopsis, where Julin has described the emergence of the centrosome from the nucleus of the spermatid, without, however, being able to trace it into the fertilisation spindle.

In the primary spermatocytes of Ascaris, Brauer (1893) has observed and figured the appearance and even the division of the centrosome, with accompanying formation of the spindle, in the interior of the nucleus.

Hertwig has shown that in the reproductive cycle of Actinosphærium, a centrosome emerges from the nucleus immediately before the polar divisions of the secondary cysts. Schaudinn has actually seen *intra vitam* the centrosome escaping from the nucleus in the spore of Acanthocystis.

Lastly, in the primary oocyte Rückert (1894) has asserted a similar origin of the centrosomes in Cyclops, while the same view has been, though more doubtfully, expressed for other forms (Cerebratulus [Coe], Thalassema [Griffin], Prosthecerœus [von Klinckowström], Myzostoma [von Kostanecki], Asterias [Matthews], Thysanozoon [van der Stricht], Polychœrus [Gardiner], and Cyclas [Stauffacher]); in all these cases the centrosomes first appear in invaginations of the membrane of the germinal vesicle.

(ii) Structure and functions of the centrosome.

The centrosome is a body which is almost invariably to be found during the division of the animal cell. There are, however, some exceptions. It is stated by Boveri (1887, 1890) to be absent from the polar spindles of Ascaris and Sagitta. Sobotta has made the same statement of the polar spindles of Amphioxus and the Mouse, Brauer and Behrens of those of Branchipus and the Trout respectively, and various authors (Carnoy, Fick, and myself) of the polar spindles of Amphibia. Further, its existence in the cells of the higher plants is totally denied by Strasburger and his school.

With regard to all these cases, I venture to make two suggestions. As far as the plants are concerned, it is only fair to say that Guignard and many other observers still adhere to the opposite view. In the second place, no one will pretend that the pole of a spindle is occupied by a Euclidian point; some small particle is undoubtedly there which may be

physiologically a centrosome, even though it refuses to stain with iron-hæmotoxylin. With respect to its alleged absence from certain polar spindles, it may be pointed out that in Ascaris it has been seen by several investigators (Carnoy, Sala, and Fürst), and that in any case the broad plate which here occupies the pole of the intra-nuclear spindle has just as much title to be regarded as active in the production of the spindle fibres as has the quite similar pole-plate in the spindles of Infusoria, Actinosphærium, and other Protozoa.

That the centrosome is not merely passive I hold to be proved, first, by its division antecedently to the formation of those structures on which the division of the nucleus and cell obviously depends; and secondly, by the fact that these structures (astral rays and spindle fibres) clearly grow out from the centrosome. Further, I think it possible that the activity depends, as Bütschli (1894) first suggested, on its faculty of absorbing the watery substances of the cytoplasm. Such absorption will readily account for its growth, and perhaps also for the remarkable series of periodically recurrent changes which it passes through.

These changes have been noticed and figured by many cytologists (Coe, Lillie, Vejdovský, MacFarland, Sobotta [Amphioxus], Conklin, van der Stricht [1898], Linville, Gardiner, Griffin, and myself); but it is to Boveri (1901) that we owe the clearest description of the details of the process.

In spite of much disagreement, especially with regard to the nomenclature of the different parts of the structure, all are at one in regarding as the essential feature of the metamorphosis (a) the enlargement of the centrosome at a certain stage in mitosis, (b) the gradual fusion of the centrosome with the aster, from which it now becomes indistinguishable, and together with which it ultimately degenerates, (c) the formation of a new centrosome inside the old; this new centrosome divides preparatory to the next mitosis, while around its halves the new asters are formed.

This is essentially Boveri's account of this cycle of changes in the fertilization spindle of Echinus. The centrosome, by

which he understands the reticular spherical body from which the rays of the aster start, grows in the anaphase and gradually merges with the aster. Meanwhile, by condensation of the central portion of the old, a new centrosome is formed, which divides, and is the starting-point for new asters and a new spindle.

In Ascaris the process is a little different. Here the centrosome enlarges until the metaphase is reached; it then begins to diminish, and continues to do so until it divides. It should be noticed that a centriole is distinctly visible in its interior throughout. What happens during its diminution may best be described in Boveri's own words: "Natürlich mussen gewisse Teile abgestossen werden; allein dieser Prozess scheint sich in den meisten Fallen so allmählich zu vollziehen dass er kaum bemerkbar ist und die abgestossene Teile nicht als solche erkannt werden konnen" (the surface of the centrosome is rough and ragged at this stage); "diese Bilder mogen mit der Auflösung peripherer Centroplasmaschichten zusammenhangen." Again he says: "Das verkleinerte Centrosom ist stets der Mittelpunkt der Radien die sich ihm unmittelbar anfügen und die offenbar aus dem abgestossenen Centroplasma gebildet sind." Finally he concludes: "Das periphere Centroplasma sich von dem centralen gesondert und ähnlich wie beim Seeigelei der Sphäre angeschlossen hat."

I think it is perfectly clear from this that Boveri regards the diminution of the centrosome in the anaphase of Ascaris as parallel to the condensation of a new centrosome in the interior of the old in Echinus. In that case the only difference between the two is this: in Echinus the centrosome grows by simple enlargement, in Ascaris it grows by giving off rays which become continuous with the older rays outside. In both cases the outer portion of the enlarged centrosome becomes indistinguishable from the aster, and together with it undergoes a granular or reticular degeneration.

The changes figured by Conklin in Crepidula are closely similar to those described by Boveri for Echinus; the same may be said of Sobotta's figures of Amphioxus, Coe's of

Cerebratulus, van der Stricht's of Thysanozoon (second polar spindle), and Griffin's of Thalassema. The cleavage centrosome of the Axolotl, on the other hand, resembles that of Ascaris. At first small, it increases in volume, and then gives off fine rays, which become continuous with the older astral rays outside. These rays then degenerate to form the centrosphere, in the middle of which a "reduced" centrosome (to use Boveri's expression) is found. This divides for the next mitosis, and, like the centrosome of Ascaris, contains a minute centriole.

Lillie describes in the maturation and fertilisation spindles of Unio an inner radiate sphere immediately outside the centrosome, between which and the aster proper is a second or outer, also radiate sphere. In the anaphase the inner sphere enlarges, while the centrosome divides, a spindle being formed between the halves. Then, while the inner sphere disintegrates together with the outer sphere and aster, each centrosome grows to form the inner sphere of the next generation, one central particle remaining as the centrosome. Lillie's inner sphere is clearly a derivative of the centrosome, and its whole history shows very clearly that a part—the outer part—of the centrosome may in the course of its life assume a radial structure. This, as pointed out above, is admitted by Boveri, and, I think, follows from my own observations.

Vejdovský's interpretation of the corresponding changes in Rhynchelmis is very different. The substance of the sphere, which is cytoplasmic in origin, assumes a radiate arrangement under the influence of the central body or centriole (he admits no centrosome). The central portion of this sphere, or centroplasm, as Vejdovský calls it, undergoes degeneration only once more to assume a radial arrangement about each half of the dividing centriole. The central body, therefore, undergoes no increase of size, and exhibits no alteration of structure. The changes are entirely confined to the surrounding cytoplasm (centroplasm), and are merely called forth by the activity of the centriole.

I cannot help thinking that a media via may be found between these two opposite views; for if, as I have suggested above, the centrosome is capable of sending out radial processes which precipitate the cytoplasm, it is quite clear that the centrosphere must be derived from one as much as from the other.

(b) The origin of the cleavage centrosomes.

The dominant theory of the origin of the cleavage centrosomes is undoubtedly that propounded by Boveri on the basis of observations on the egg of Ascaris. It is this: the egg lacks the organ of cell division, the centrosome; this is supplied in the act of fertilisation by the spermatozoon.

How powerful the influence of this conception has been on the interpretations which subsequent investigators have put upon their work s patent to anyone who is acquainted with the literature of the subject, and is seen in the frequency with which the identity of the cleavage with the sperm-centrosomes is asserted on purely à priori grounds when positive evidence is wanting.

On the other hand, there have been a few who have been content to leave the origin of these organs undetermined, while a very few either deny the participation of the sperm-centres in the formation of the fertilisation spindle altogether, or at least assert that the egg centres also play a part in the process.

Lastly, an attempt has been made, in extension of Boveri's original hypothesis, to prove the persistence of the centrosome of the spermatid as the sperm- and consequently as the cleavage-centre.

These various hypotheses I propose to examine separately.

(i) The participation of the egg centres in the formation of the cleavage spindle.

While the majority of investigators agree in asserting the disappearance of the egg centrosomes and asters after the

formation of the second polar body (Castle [Ciona], Coe and von Kostanecki [Cerebratulus], Griffin [Thalassema and Zirphæa], Foot [Allolobophora], Lillie [Unio], etc.), Wheeler has stated that in Myzostoma not only do they persist but alone are concerned in the production of the fertilisation spindle. "I have never been able," says this author, "to find any traces of such archoplasm or any centrosome in connection with the male pronucleus." This account is, however, contradicted by von Kostanecki (1898), who, while fully admitting the prolonged persistence of the egg-centres, claims to have discovered two centrosomes in proximity to the sperm-nucleus, and to have seen the formation of the fertilisation spindle from these. He admits, however, that the verdict must ultimately be given on "die Analogie mit dem Befruchtungsvorgang bei anderen Thierspecies."

While no one except Wheeler has denied to the sperm-centres some share in fertilisation, Conklin and others have revived Fol's almost forgotten "Quadrille des centres." Conklin described this in Crepidula, but in a subsequent paper contradicted his earlier account. His later view is that the sperm and egg-asters fuse and that then the combination-aster divides, the cleavage centrosomes arising within the daughter-asters in a manner which is not further determined. Blanc has made a somewhat similar assertion for the Trout, but he is contradicted by Behrens; while van der Stricht's figures of the "Quadrille" in Amphioxus are shown by Sobotta to be really taken from polyspermatic ova.

(ii) Origin of the cleavage centrosome not determined.

In Arenicola (Child), Allolobophora (Foot), Pleurophyllidia (MacFarland), Unio (Lillie), Prostheceræus (von Klinckowström), Polystomum (Halkin), Insects (Henking), and Cerebratulus (Coe), the sperm-asters and centres disappear; the cleavage centrosomes then arise de novo. In some cases (Cerebratulus, Allolobophora, Unio) they are first

seen at the poles of the united pronuclei, and Lillie surmises that one is derived from each. Others (Coe, MacFarland) conjecture that they must, nevertheless, be considered to come from the sperm.

(iii) Origin of the cleavage-, from the sperm-centres.

The remaining authors express themselves more positively, and in some cases the evidence is perfectly good. It is so, for example, in the Axolotl, in Cyclops (Rückert [1895]), Diaptomus (Ishikawa), Branchipus (Brauer [1892]), Rhynchelmis (Vejdovský), Ophryotrocha (Korschelt), Toxopneustes (Wilson), Ciona (both Castle and Boveri).

In Chætopterus and Thalassema, again, Mead and Griffin assert most categorically the continued existence of the sperm-centrosomes, but in Cerebratulus and Physa and in the Mouse the sperm-centres disappear, and von Kostanecki and Sobotta are constrained to fall back on à priori considerations in order to establish their identity with the definitive centrosomes.

In other cases there is less certainty (Polyclada [Francotte], Petromyzon [Böhm], Amphioxus [Sobotta]), and even in Ascaris Boveri (1888) was unable to do more than state what was in his opinion the very great probability of the introduction of the cleavage centres by the spermatozoon. Von Erlanger has, however, since shown that this was justified by demonstrating the presence of a centrosome in the spermatozoon, and its division to form the centres of the fertilisation spindle.

(iv) The persistence of the centrosome of the spermatid as the sperm- and cleavage-centre.

It is true that the most recent investigations agree in tracing the centrosome of the spermatid into the middle-piece of the spermatozoon. At the same time the sperm-

centre is first seen in the majority of cases on the inside of the sperm-nucleus. In this case, its origin from the middle-piece cannot be said to have been demonstrated.

The rotation of the sperm-head has, however, been observed in Toxopneustes, the Trout (Behrens), Petromyzon (Herfort), Sponges (Maas), Ophrytrocha, Branchipus; while the formation of an aster round the middle-piece is recorded for Polyclada (Francotte and von Klinckowström), Allolobophora, Physa, Crepidula, Petromyzon, Rhynchelmis, Toxopneustes, Ascaris, the Axolotl, and the Newt. Miss Foot and Wilson, however, assert that in Allolobophora and Toxopneustes the middle-piece disappears and stands in no obvious genetic relation to the cleavage centrosomes.

It is, perhaps, a matter of little moment that the middle-piece should have been traced to the previous centrosome in none of these cases except the Axolotl; what is of importance is that the formation of an aster about this structure is no indication whatever of its survival as the cleavage centrosome, as its fate in the Axolotl and Newt, in Allolobophora and Toxopneustes clearly shows.

The difficulty of drawing any positive conclusion from this conflicting mass of testimony is obviously very great; for as Wilson has pointed out, if the sperm-centres disappear there is no more reason for deriving the cleavage centres from them than from the egg-centres. The possibility of the formation of centrosomes afresh in the cytoplasm has also to be reckoned with (Mead, centrosomes in the oocyte of Chætopterus; Wilson [1901] and Morgan, centrosomes in the parthenogenetic ova of Echinoderms).

It would be unwise to prophesy too dogmatically until we have a much fuller knowledge of the exact mode of formation of the cleavage centres; but it does not seem impossible that they may arise in other forms, as they do in the Axolotl, from the sperm-nucleus; and that those sperm-asters which have so often been observed, and so often disappear, are the transitory primary radiations which arise around the middle-piece. By giving up therefore the doctrine

of the continued persistence of the centrosome from the spermatid to the completely fertilised ovum, we may be taking the first step towards re-establishing on a securer basis Boveri's original generalisation.

The rehabilitated theory of the prime activity of the spermatozoon in renewing the ovum's lost power of cell-division might then be enunciated as follows :—On contact with the egg an apparatus—the entrance-cone—is produced for ensuring the entrance of the sperm; the organ responsible for this is the acrosome. In the interior of the egg a sperm-sphere appears which imparts (as Ziegler's experiment has shown) a second stimulus to the cytoplasm; the organ which is now concerned is the middle-piece. When the pronuclei have met a spindle, formed directly by the divided sperm-centrosome, completes the process of nuclear and cell-division. Since, however, all these three organs either are, or are derived from centrosomes, the supreme physiological importance of the centrosome in the act of fertilisation is vindicated to the full.

IV. Experimental.

In this section I propose to give a brief account of some experiments I have made in the hope of throwing some light on the nature of the physical processes concerned in the act of fertilisation, that is to say in the restoration to the ovum of its lost power of cell-division.

We have seen that not only in the Axolotl, but also in a large number of other forms the following phenomena have been observed during fertilisation :—

1. The formation round the spermatozoon of an entrance-funnel filled with a plug—the entrance-cone—consisting of some coagulable, apparently watery material.

2. (a) The appearance of a clear area devoid of yolk-granules round the sperm-head and middle-piece when the latter has reached the interior of the egg.

(b) The vacuolation of this clear area and simultaneous

assumption by it of a radial structure, the rays being prolonged outside it between the surrounding yolk-granules.

(c) The formation of a spindle between the centrosomes, accompanied by a great increase in volume of the latter.

In considering these two classes of phenomena I could hardly refrain from indulging in vague conjectures in explanation of them, and it was with a view to testing these speculations that I undertook the two sets of experiments now to be described. As a result, I have been tempted to form certain conclusions; but I must state most explicitly that the experiments are themselves very far from being thorough or searching, and that the hypotheses founded on them are tentative in the very highest degree.

1. It occurred to me that the entrance of the spermatozoon with the accompanying formation of entrance-cone and funnel might be due to a local alteration of the surface tension of the egg. I floated a fairly large drop of acetic acid between a layer of chloroform and a layer of benzole in a glass vessel. The drop assumed approximately a spherical shape. In the same vessel I floated a drop of filtered albumen. When the drops were made to touch and coalesce the acetic seemed to spread over the outer surface of the albumen; and this was very clearly the case when the drop of acetic was much smaller than the other, the acetic producing a patch of coagulum on the outer surface of the albumen. I concluded from this that the surface tension between acetic and the mixture of chloroform and benzole was less than that between albumen and the mixture. I then took a large drop of acetic and a small drop of albumen; in this case, when the drops coalesced the smaller streamed into the interior of the larger.

Exactly the same thing occurred when I substituted for the albumen either a drop of gum or a drop of a semi-solid mixture of 1 per cent. gelatin and albumen in equal parts. The shape of the instreaming drop varied, however, in the three experiments. In the case of the albumen the inner end was broader than the outer, with the gum the drop streamed in as a

cylinder, while the gelatin-albumen preserved its spherical form.

I suggest, therefore, merely of course as a working hypothesis, that the entrance-cone—the plug of apparently watery substance which fills up the entrance funnel—is in reality the agent which produces this deep depression at the surface of the egg, and carries the spermatozoon with it into the interior; and that it does so in virtue of its greater surface tension. We should expect then a more watery proteid like albumen to behave toward a less watery one such as egg-yolk as the albumen behaves toward the acetic acid; and this is in fact the case. A small drop of albumen will enter a large drop of egg-yolk, while conversely a small drop of yolk spreads over the surface of a large drop of albumen.

The substance with the greater surface tension is of course derived from the egg itself. It appears only when the spermatozoon comes in contact with the egg, and we must therefore ascribe to the male cell the important function of withdrawing water from the cytoplasm. It is further probable that this intense hygroscopic activity may be located in a particular organ of the spermatozoon, the acrosome; Miss Foot and Vejdovský have indeed already suggested that this is the active agent in the production of the entrance-cone. In this connection it is of the greatest interest that Meves should have described the origin of the acrosome in the salamander and guinea-pig from the sphere of the spermatid, a body related most intimately to the centrosome; for, as I believe, and as I hope the experiments next to be described may show, the activity of the centrosome also depends very largely on its power of absorbing water from the cell.

2. The second series of experiments starts from the observed concentration of a watery substance in the centre of the sperm-sphere.

I began by placing a small crystal of ammonium sulphate in a drop of filtered albumen on a slide. As the crystal begins to dissolve a pool or vacuole of its own solution is formed immediately round it, and outside this there quickly

develops a system of bright, radiating lines. These lines appear equally well whether the preparation is covered by a glass or not. I soon came to the conclusion that the bright lines were tracts of albumen left between tubular outgrowths of the vacuole, though it is not very easy to make this out in this particular experiment. In other cases, however, to be described in a moment, this can be clearly seen to be so. If carmine particles are placed in the albumen they may be observed to stream towards the crystal.

As the crystal continues to dissolve the solution approaches the saturation point; a thick brown ring or wall of precipitated albumen is then formed round the central vacuole. Through this, however, the solution passes, and there are produced outside the wall a number of fine rays of precipitate. This "diffusion aster," as I will term it to distinguish it from the other or "excurrent aster," is of course identical with the structure described by Fischer under the title of "Selbststrahlung." Both kinds of aster are transitory and soon dissolve in the albumen.

The experiment may be varied by using instead of the ammonium sulphate crystal a drop of glycerin, or glycerin and albumen, or glycerin and sublimate; or again a small particle of dried gum, the gum being either used pure or with the previous admixture of potassium carbonate, picric acid, or ammonium sulphate. The result is the same except that when the substance employed is a precipitating reagent the radiating lines of albumen between the tubular outgrowths become fixed. With some substances, chromic for example, I found that only the diffusion aster could be produced.

I found subsequently that very much better results could be obtained by employing a thin layer of albumen; using these, beautiful asters can be made with a drop of sublimate, picric, or ammonium sulphate. Although the layer of albumen is exceedingly thin, still I believe that even here the outgrowths take place in the thickness of the film; for the drop spreads before the radial outgrowths are given off from its periphery, and an upper membrane of precipitate can be

lifted off the lower layer which forms the floor of the central circular area.

I next tried gelatin, principally a solution of about 6 per cent., and succeeded in producing the excurrent aster with picric acid, either alone or with the admixture of glycerin or cane sugar; with chromic acid and glycerin, and with Flemming's solution; with albumen mixed with either glycerin or cane sugar; with a crystal of either ammonium sulphate or sodium chloride, and with saturated solutions of either substance; and with a mixture of gum and sublimate. As before the results are far superior when a thin layer of gelatin is used. The asters retain their form long enough for the gelatin to set; they may then be fixed in alcohol and preserved permanently.

Thirdly I experimented with yolk of egg. If a small crystal of ammonium sulphate be immersed in a drop of egg-yolk, it does not matter how large or thick, a clear area is at once formed round it, the yolk-granules being driven away. This can be seen in the drop and is easily verified by the aid of sections. Soon there appears internally to this clear area a thick brown wall of precipitate, as in the case of albumen described above, and inside this a central vacuole as the crystal finally dissolves away.

If instead of ammonium sulphate a small crystal, the smaller the better, of salt or sugar be employed no precipitate is formed, but short radial tubes grow out into the clear zone from the central vacuole, and not only in a horizontal plane. It is important to observe that these outgrowths can be produced as easily in a large drop as in a small, and that in the former case their formation is quite independent of any contact with the lower surface of the drop next the glass.

If on the other hand a thin film of egg-yolk be employed the aster is much more fully developed. In egg-yolk I have made asters with solutions of picric, picric and cane sugar, cane sugar, glacial acetic, aceto-corrosive, chromic, chromic and acetic, glycerin and sublimate, glycerin and picric, ammonium sulphate and 90 per cent. alcohol. The best results are given by glacial acetic and cane sugar.

As the process takes place much less rapidly here than in other cases the formation and structure of the aster may be very readily observed. The drop spreads out in the thickness of the film; radial processes are then given off from its circumference, which as they grow out branch repeatedly and anastomose with one another. In this way tracts of egg-yolk left in between the excurrent radii may be cut off and isolated from one another. Where the radii leave the central drop, and where their branches leave the radii, they are frequently exceedingly narrow; in their formation the contained liquid first pierces a small aperture in the surface (or surface membrane) between itself and the yolk, and then expands on the outer side. The intervening portions of yolk are naturally thickened here and often fuse with one another, pieces of the excurrent radii being thus cut off in their turn. In this way the whole aster comes to have the appearance of a system of radially elongated alveoli, more or less completely separated from one another by thin intervening lamellæ. When two such asters are formed close together and simultaneously, a spindle results with a plane equatorial plate where the opposing radii meet (fig. D.). The aster is frequently made up of concentric zones; this is due to the radii branching, and rebranching at equal distances from the centre.

Lastly, asters of the same type were made with many of the above-mentioned reagents in mixtures of gum and gelatin and of gum and albumen.

My next efforts were directed towards producing these outgrowths in the bulk of the colloid, and here I have been less successful.

The following experiments were tried:—A small drop of dried gum saturated with potassium carbonate was supported on a needle-point in a vessel of filtered albumen. Tubular processes were given off in all directions, but soon turned down and sank to the bottom. In albumen, however, which has become highly viscid by desiccation, the tubes which are given off retain their original direction.

A drop of picric acid was placed in a $\frac{1}{2}$ per cent. cold solu-

tion of gelatin; whether this solution is wholly liquid or contains solid matter I must leave it to the physicists to decide, but it seemed to me to be a fluid containing some solid in suspension. The picric acid sinks but slowly, and gives off tubes in the bulk of the fluid.

In a $\frac{2}{3}$ per cent. solution of gelatin set to a jelly, which, as Hardy has shown, contains liquid and solid side by side,

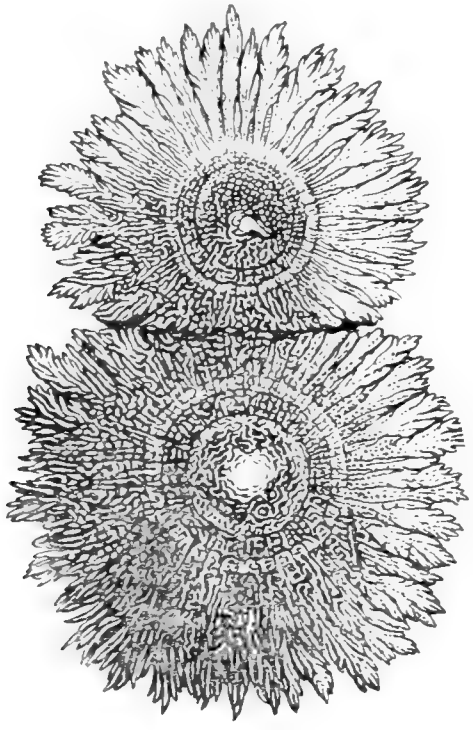

FIG. D.—Photograph of an artificial spindle made with glacial acetic acid in a film of egg-yolk on a slide. Note the equatorial plate.

drops of 1 per cent. chromic and saturated ammonium sulphate sink partially below the surface; radial tubes are given off in all directions from the underside of the drops. Other substances give results; they are not, however, nearly so good.

This led me to make a few experiments with fluids in which solid particles are suspended. I have tried albumen

beaten up but unfiltered (which of course contains much solid matter), a mixture in equal parts of 1 per cent. gelatin and albumen, and filtered albumen mixed with a little yolk of egg. With the first both picric and metaphosphoric acid (about 1 per cent.) will give off radial tubes in the bulk of the liquid; with the second, gum and picric, metaphosphoric acid and crystals of salt and ammonium sulphate; with the third, metaphosphoric acid. I did not make a very extended series of trials.

In none of these cases could I succeed in obtaining such fine asters as in thin films and on a glass slide; and I always observed that the tubular outgrowths developed much more rapidly when they could run along the under side of the surface of the fluid.

The difficulty of getting the tubes to grow out in the bulk of the liquid depends no doubt in part on the difference in specific gravity of the two substances employed, the drop always sinking to the bottom before it has time to send out its processes. It is, however, due, I believe, in much larger measure to the absence of certain very essential physical conditions.

It will have been noticed that the reagents selected for the production of these artificial asters are, with the exception of gum, all crystalloid, and possessed therefore of a far higher osmotic pressure than the colloidal solutions in which they are placed. They were indeed chosen for this very reason; for I was under the impression that we had here to do simply with phenomena of osmosis, and that the tubular outgrowths were merely due to an excess of pressure on the inside. I believed, in fact, that the behaviour of the ammonium sulphate crystal in albumen was strictly comparable to the behaviour of a crystal of potassium ferrocyanide in a solution of copper sulphate. In this experiment (for which I am indebted to Dr. Ramsden) a colloidal membrane of copper ferrocyanide is rapidly formed round the crystal as it dissolves, from which membrane numerous irregular twisted tubes grow out in all directions.

This, however, is by no means the case; for in the first place a drop of distilled water will produce an aster in egg-yolk or albumen; and further, the asters can be made much more readily, as already pointed out, on a glass slide and in a thin film, or at the surface of a liquid, than in the bulk of a liquid, and in the latter much better when there are solids present.

It is quite evident then that though a central excess of osmotic pressure may be to a certain extent responsible for the production of the aster, surface-tension phenomena of a very complicated nature have still to be reckoned with. More than this as to the physical nature of the process it is impossible to say. There seems to be an important difference between these asters and the well-known "cohesion figures" of Tomlinson. No doubt both are capillary phenomena, but while Tomlinson's figures are formed at the surface these grow out beneath it in the thickness of the film. Surface-tension relations with both air and glass are thus apparently excluded. My asters also are quite dissimilar to the "strain" asters produced by Bütschli (1898) in gelatin under the stress of a contracting air-bubble, and made by Hardy with a small globule of mercury rolled on a thin film of albumen. Dr. Ramsden has pointed out to me that the latter is nothing more than the wrinkling of a solid surface membrane, and can hardly be compared with any radiations formed in the bulk of a fluid.

It only remains to be considered whether any hypothesis, however tentative, can be based on these experiments which shall elucidate the natural asters which we observe in the living cell.

We have seen that when the spermatozoon reaches the interior of the ovum a clear yolk-free area is formed round it, in the centre of which the middle-piece gradually dissolves. The behaviour of the middle-piece in the egg seems quite comparable with the behaviour of a small crystal of salt or other substance in a drop of egg-yolk; here also a clear yolk-free area is formed round the dissolving particle.

Subsequently the sperm-sphere assumes a radiate structure. I suggest that this structure is due to the outgrowth of tubular processes from the central dissolved mass. These outgrowths, filled with a slight coagulum, constitute the alveoli or inter-fibrillar spaces; the intervening tracts of the substance of the sphere the inter-alveolar lamellæ or fibres.

In addition to these rays, however, other rays are formed, passing outwards between the yolk-granules. These external rays I must regard as originating by a different process; I believe that they represent the paths along which water is being withdrawn from the cytoplasm. Bütschli (1894) has described such rays round the contractile vacuole of Balantidium and some other Protozoa. The water thus continually withdrawn from the egg becomes concentrated in the large vacuoles which we have seen occupying the centre of the sphere.

It is at this moment that the definitive centrosome makes its appearance. Its probable origin through precipitation of the albumins of the egg-cell by the nucleic acid or nucleins of the sperm-nucleus has already been discussed. It has also been shown that the spindle-fibres appear to grow out from the centrosomes, and that as the spindle is gradually developed so the centrosomes gradually enlarge. It seems to me that the physical interpretation suggested above of the formation of the sperm-aster is applicable here also, only that the active hygroscopic particle is now the centrosome instead of the middle-piece. Accepting this view, we regard the spindle-fibres and such parts of the astral rays as come into being at this stage as inter-alveolar lamellæ, the alveoli themselves as outgrowths of the dissolved substance of the centrosome. The intra-nuclear portion of the spindle arises by the extension of the tubular outgrowths into the cavity of the nucleus, the membrane being first dissolved. The fibres are then formed from tracts of achromatic substance, just as outside they are formed from the cytoplasm.

Assuming that the centrosome—and the middle-piece is also a centrosome—contains nucleic acid or even nuclein we have in it an agent capable of producing these effects; meta-

phosphoric acid, a characteristic constituent of the nucleins (Mann) has already been mentioned as one of the reagents used in the production of the artificial asters; and Berg has shown that the precipitation granules produced by the action of nucleic acid and nuclein on clupein, a protamin, are capable of swelling up with the water they absorb. Further, since, as is well known, nucleic acid and nuclein precipitate albumins—in virtue apparently of this same metaphosphoric acid—we shall, on the hypothesis I am advocating, have to regard the spindle-fibres as solid or at least as solid as these proteid precipitates usually are. That the spindle has a considerable amount of rigidity seems to be shown by the fact observed by Gardiner and Vejdovský that it does not readily change its shape even when the egg is deformed or burst.

The spindle-fibres are then primarily lamellæ lying between radial tubes running out from the centrosome and consisting of a precipitate of the albumins of the cell (or nucleus) by the nucleins in solution in the tubes; by the anastomosis of adjacent outgrowths the lamellæ may become converted into actual fibres; while the concentric zones of the real asters are produced, as they are in the artificial, by the branching of the outgrowths at points equidistant from the centre. Where two such radial systems meet a spindle is formed, the chromosomes being pushed into the equator; if the opposed ends of the radial tubes fuse bi-polar fibres will result, if they inter-digitate, fibres intercrossing at the equator, if they meet but do not fuse, an achromatic equatorial plate. This condition may be easily imitated (Fig. D.). In the anaphase of the fertilisation spindle of the Axolotl I have described such a plate; but there is an earlier stage in which the fibres pass continuously from pole to pole. I think this may be explained as follows: I have often observed that the outer end of the artificial tubes are covered only by an extremely thin membrane, apparently because the concentration of the liquid inside is too low to produce a copious precipitate. Such thin-walled ends would readily fuse, but as the concentration increased at this point the dissolved proteids would be reprecipitated.

I have also a word to say on the so-called contractile fibres or "Zugfasern" attached to the chromosomes.

In the Axolotl I have seen such fibres, or rather fibre-bundles, passing to but not beyond the chromosomes; as the latter diverge the fibre-bundles shorten, though they cannot be said to thicken. Usually the fibre-bundle is attached to the end of a chromosome but sometimes at a short distance from the end. In this case the point of attachment is during the anaphase invariably nearest the spindle-pole, the chromosome thus assuming a hooked form. This all seems to me to be strongly in favour of the belief that these fibre-bundles do actually pull the chromosomes apart. There is of course a large amount of evidence to the same extent from many other sources. At the same time I believe it to be a wholly gratuitous error to attribute to such fibres the properties of pieces of elastic, as so many authors have done, or to assume with Boveri (1888) that all the laws that hold good for muscles can also be applied to these.

On the view I have put forward these fibres, produced by the precipitation of a proteid, are probably in the condition of a highly viscous fluid. When a drop of egg-yolk falls from a glass rod it draws out a long thread behind it; when the drop is detached the thread flows back on to the rod. And so in the spindle. As the tubes grow out some of the lamellæ, or fibres, become attached to the chromosomes; when the chromosomes split the fibres retreat into the substance of the centrosome, carrying the halves of the chromosomes with them. The astral rays on the other hand do not behave in this way, probably because their outer ends never become severed from the surrounding cytoplasm.

Cases have been described (Iijima, Mark) in which the astral rays are curved, apparently by streaming movements in the cell. Such a curvature may easily be imparted to the artificial radiations by simply tilting the slide. It is very difficult to believe that these rays are any more elastic than the spindle-fibres.

Lastly, the living aster and spindle dissolve and disappear

in the cytoplasm in exactly the same way as, for example, the ammonium sulphate aster is resoluble in an excess of the surrounding albumen.

My theory then of the formation of these structures which appear in the egg during fertilisation is that they are produced under the influence of the middle-piece and centrosome in virtue of a capacity which these bodies possess of withdrawing water from the cytoplasm,[1] of swelling up and dissolving in the water so absorbed, and then giving off radial outgrowths which precipitate the proteids of the cell so producing an aster and, by the combined effect of two, the fertilisation spindle.

I am therefore very closely in accord with those authors who like Meves (1896, 1898) see in such facts as the invagination of the nuclear membrane, the divergence of the centrosomes and the broadening of the spindle, strong grounds for holding that spindle-fibres and astral rays are structures which grow out from the centrosome. The difference between us is that according to my theory it is not the fibres, but the inter-fibrillar spaces or alveoli which are the more actively concerned in the process. Not that I regard all asters as necessarily formed in this way. It is quite probable that in many cases asters may be precipitated by the centrosome in the manner termed "Selbststrahlung" by Fischer. Most authors of course figure asters of this type, that is, systems of radiating disconnected straight lines.

On the other hand I stand in absolute opposition to those who regard rays and fibres as permanent organs of the cell, and whose whole cytological philosophy is summed up in the dogma "Omnis radius e radio." Such theories ignore the periodic disappearance and re-formation of these structures,

[1] Dr. Ramsden has suggested to me that the centrosome may not only be hygroscopic, but may either itself undergo decomposition or possess a ferment which would produce such an effect on the cytoplasm. In either case the result would be an increase in the number of molecules, that is, in the osmotic pressure. This might be partly responsible for the formation of the aster (see above).

and, when they apply the theory to the explanation of cell-division, the very obvious fact that in many cases these "elastic" threads never reach the surface of the cell at all.

Neither can I agree that the centrosome is passive, the mere "Insertionsmittelpunkt" of contractile fibrillæ. In spite of the asserted absence of the centrosome in the higher plants—and we shall do well to remember that the question is still sub judice and that much depends on our definition of a centrosome—and in spite of the difficulties presented by the facts of multi-polar mitosis, I confess I am one of those who believe in the centrosome as active—whether permanent or not is of little moment—and as active because it is hygroscopic. This conception of the centrosome as an absorbent of the water of the cell is of course not new. Bütschli (1894) suggested that it had this function and showed that in his artificial foams a radial structure might be induced round a central hygroscopic particle. But here our paths diverge. For Bütschli an alveolar structure is appropriate to all living substance and the aster we see is but the radial rearrangement of the alveoli that existed before. The theory has grave objections. In the first place an assumption is made as to the structure of protoplasm, an assumption which has not yet been vindicated; and in the second no explanation is offered of the manner in which an aster so produced could perform its functions.

On the other hand while the theory which I have ventured to put forward asks for no other preconception of the nature of living substance than that it is a colloidal fluid, it does, I hope, indicate a way in which those structures which we do really see may not only be formed, but also be capable of effecting the observed results, as far at least as the division of the nucleus goes. (The division of the centrosome is another matter entirely.) This way is by the redistribution of the watery contents of the cell, and should this lead to a disturbance of the equilibrium of internal surface-tensions a way may be opened for the explanation of cell-division as well. The facts of normal fertilisation might thus be brought

completely into line with the phenomenon of artificial parthenogenesis, a phenomenon which, as is well known, Loeb has attributed to the increased osmotic pressure of the medium in which the eggs are placed.

But whether this withdrawal of water is or is not the essential factor in the formation of the wonderful structures we observe in fertilisation, whether my tentative hypothesis may usefully serve as a light to lighten the path of other investigators, or whether it is destined to be cast into the outer darkness of misguided speculations, I hope that it may at least show the urgent necessity of supplementing the descriptive by the experimental study of developmental processes; for until that is done we can make no profitable progress, nor can our theories claim to be scientific in the fullest sense of the word.

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List of Works referred to in the Text.

Behrens, G.—"Die Reifung und Befruchtung des Forelleneies," 'Anat. Hefte,' x, 1898.

Berg, W.—"Beiträge zur Theorie der Fixation," 'A. m. A.,' lxii, 1903.

Blanc, H.—"Étude sur la fécondation de l'œuf de la Truite," 'Ber. d. Naturf. Gez. su Freiburg i. B. Festschr. f. Weismann,' 1894.

Böhm, A. A.—"Ueber Reifung und Befruchtung des Eies von Petromyzon Planeri," 'A. m. A.,' xxxii, 1888.

Born, G.—"Die Struktur des Keimbläscheus im Ovarialei von Triton tæniatus,' 'A. m. A.,' xliii, 1894.

Boveri, T.—"Zellen-Studien I. Die Bildung der Richtungskörper bei Ascaris megalocephala und A. lumbricoides," Jena, 1887.

Boveri, T.—"Zellen-Studien II. Die Befruchtung und Teilung des Eies von Ascaris megalocephala," Jena, 1888.

Boveri, T.—"Zellen-Studien III. Ueber das Verhalten der Chromatischen Kernsubstanz bei der Bildung der Richtungskörper und bei der Befruchtung," Jena, 1890.

Boveri, T.—"Zellen-Studien IV. Ueber die Natur der Centrosomen," Jena, 1901.

Brauer, A.—"Ueber das Ei von Branchipus Grubii von der Bildung bis zur Ablage," 'Phys. Abhandl. k. Ak. Wiss., Berlin,' 1892.

BRAUER, A.—"Zur Kenntniss der Spermatogenese von Ascaris megalocephala," 'A. m. A.,' xlii, 1893.

BRAUS, A.—"Ueber Zelltheilung und Wachsthum des Tritoneneies,' 'Jenaische Zeitschr.,' xxix, 1895.

LE BRUN.—"La vésicule germinative et les globules polaires chez les Anoures," 'La Cellule,' xix, 1901.

LE BRUN.—"Les cinesès sexuelles chez Diemyctilus torosus," 'La Cellule,' xx, 1902.

BÜTSCHLI, O.—"Investigations on Microscopic Foams and on Protoplasm," English Translation, London, 1894.

BÜTSCHLI, O.—"Untersuchungen über Strukturen," Leipzig, 1898.

CARNOY et LE BRUN.—"La vésicule germinative et la globule polaire chez les Batracieus," 'La Cellule,' xii, xiv, 1897, 1898.

CARNOY et LE BRUN.—"Les globules polaires des Urodèles," 'La Cellule,' xvi, 1899.

CASTLE, M. E.—"The Early Embryology of Ciona intestinalis," 'Bull. Harvard Mus.,' xxvii, 1895—1896.

CHILD, C. M.—"The Maturation and Fertilisation of the Egg of Arenicola marina," 'Trans. New York Ac. Sc.,' xvi, 1897, 'Referat of Fick R., Zool. Centralbl.,' vi, 1899.

COE, W. R.—"The Maturation and Fertilisation of the Egg of Cerebratulus," 'Zool. Jahrb. (Anat.),' xii, 1899.

CONKLIN, E. G.—"The Fertilisation of the Ovum (Crepidula)," 'Biol. Lect. Woods Holl.,' 1894.

CONKLIN, E. G.—"The Embryology of Crepidula," 'Journ. Morph.,' xiii, 1897.

CONKLIN, E. G.—"Centrosome and Sphere in the Maturation, Fertilisation and Cleavage of Crepidula," 'Anat. Anz.,' xix, 1901.

CRAMPTON, H. E.—"Observations upon Fertilisation in Gasteropods," 'Referat in Zool. Anz.,' xx, 1897.

CRAMPTON, H. E.—"Fertilisation of Molgula," quoted by Lillie, F. R., 'Journ. Morph.,' xvii, 1901, p. 250.

EISEN, G.—"The Spermatogenesis of Batrachoseps," 'Journ. Morph.," xvii, 1901.

VON ERLANGER, R.—"Ueber die Befruchtung und erste Teilung des Ascariseies," 'A. m. A.,' xlix, 1897.

FICK, R.—"Ueber die Reifung und Befruchtung des Axolotleies," 'Z. w. Z.,' lvi, 1893.

FICK, R.—"Mitteilung über die Eireifung bei der Amphibien," 'Verh. Anat. Ges.,' 1899.

Fischer, A.—"Fixirung, Färbung und Bau des Protoplasmas," Jena, 1899.

Foot, K.—"Preliminary Note on the Maturation and Fertilisation of the Egg of Allolobophora foetida," 'Journ. Morph.,' ix, 1894.

Foot, K.—"The Origin of the Cleavage Centrosomes," 'Journ. Morph.,' xii, 1897.

Foot, K., and Strobell, E. C.—"Photographs of the Egg of Allolobophora foetida," 'Journ. Morph.,' xvi, 1900.

Francotte, P.—"Recherches sur la maturation, fécondation et la segmentation chez les Polyclades," 'Arch. Zool. Exp.,' iii, 6, 1898.

Francotte, P.—"Recherches sur la maturation, fécondation et la segmentation chez les Polyclades," 'Mém. cour. et mém. des sav. étr.,' 'Ac. Roy. des sc. de Belg.,' lv, 1898.

Gardiner, E. G.—"The Growth of the Ovum, Formation of the Polar Bodies, and the Fertilisation in Polychoerus caudatus," 'Journ. Morph.,' xv, 1899.

Garnault, P.—"Sur les phénomènes de la fécondation chez l'Helix aspersa et l'Arion empiricorum," 'Zool. Anz.,' xi, xii, 1888, 1889.

Goldschmidt, R.—"Untersuchungen über die Eireifung, Befruchtung und Zelltheilung bei Polystomum integerrimum," 'Z. w. Z.,' lxxi, 1902.

Griffin, B. B.—"Studies on the Maturation, Fertilisation, and Cleavage of Thalassema and Zirphæa," 'Journ. Morph.,' xv, 1899.

Halkin, H.—"Recherches sur la maturation, la fécondation, et le développement du Polystomum integerrimum," 'Arch. de Biol.,' xviii, 1902.

Hardy, W. B.—"On the Structure of Cell-protoplasm," 'Journ. Phys.,' xxiv, 1899.

Henking, H.—"Untersuchungen über die ersten Entwickelungsvorgänge in den Eiern der Inseckten," 'Z. w. Z.,' xlix, 1890.

Henneguy, L. F.—"Sur la formation de l'œuf, la maturation, et la fécondation de l'oocyte chez le Distomum hepaticum," 'Comptes Rendus de l'Acad. Sc. Paris,' cxxxv, 1902.

Herfort, K.—"Die Reifung und Befruchtung des Eies von Petromyzon fluviatilis," 'A. m. A.,' lvii, 1901.

Hill, M. D.—"Notes on the Fecundation of the Egg of Sphærechinus granularis, and on the Maturation and Fertilisation of the Egg of Phallusia mammillata," 'Q. J. M. S.,' xxviii, 1895–6.

Iijima, I.—"On the Origin and Growth of the Eggs and Egg-strings in Nephelis, with some Observations on the 'Spiral Asters,'" 'Q. J. M. S.,' xxii, 1882.

ISHIKAWA, C.—"Spermatogenesis, Oogenesis, and Fertilisation in Diaptomus," 'Journ. of Coll. Sci. Imp. Univ., Tokyo,' v, 1893.

JULIN, C.—"Structure et développement des glandes sexuelles; ovogenèse, spermatogenèse, et fécondation chez Styelopsis grossularia," 'Bull. Sc. de la Fr. et de la Belg.,' xxv, 1893.

KING, H. D.—"The Maturation and Fertilisation of the Egg of Bufo lentiginosus," 'Journ. Morph.,' xvii, 1901.

VON KLINCKOWSTRÖM, A.—"Beiträge zur Kenntniss der Eireifung und Befruchtung bei Prostheceraeus vittatus," 'A. m. A.,' xlviii, 1897.

KÖLLIKER, A.—'Gewebelehre des Menschen,' 6te Aufl., 1889, p. 51 sqq.

KORSCHELT, E.—"Ueber Kerntheilung, Eireifung und Befruchtung bei Ophryotrocha puerilis," 'Z. w. Z.,' lx, 1895.

KORSCHELT, E., und HEIDER, K.—'Lehrbuch der vergleichenden Entwickelungsgeschichte der wirbellosen Thiere: Allgemeinertheil,' Jena, 1902.

VON KOSTANECKI, K., und WIERZEJSKI, A.—"Ueber das Verhalten der sogen. achromatischen Substanzen im befruchteten Ei (Physa fontinalis)," 'A. m. A.,' xlvii, 1896.

VON KOSTANECKI, K.—"Die Befruchtung des Eies von Myzostoma glabrum," 'A. m. A.,' li, 1898.

VON KOSTANECKI, K.—"Ueber die Reifung und Befruchtung des Eies von Cerebratulus marginatus," 'Bull. Intern. de l'Ac. des Sc. de Cracovie,' 1902.

LILLIE, F. R.—"The Organisation of the Egg of Unio, based on a Study of its Maturation, Fertilisation, and Cleavage," 'Journ. Morph.,' xvii, 1901.

LINVILLE, H. R.—"Maturation and Fertilisation in Pulmonate Gasteropods," 'Bull. Harvard Museum,' xxxv, 1899—1900.

LOEB, J.—"On the Artificial Production of Normal Larvæ from the Unfertilised Eggs of Arbacia," 'Am. Journ. Phys.,' iii, 1900.

LOEB, J.—"Further Experiments on Artificial Parthenogenesis," 'Am. Journ. Phys.,' iv, 1901.

LOEB, J.—"Further Experiments in Artificial Parthenogenesis in Annelids (Chætopterus)," 'Am. Journ. Phys.,' ibidem.

MAAS, O.—"Ueber Reifung und Befruchtung bei Spongien," 'Anat. Anz.,' xvi, 1899.

MACFARLAND, F. M.—"Cellulare Studien an Mollusken-Eiern," 'Zool. Jahrb. (Anat.),' x, 1897.

MCGREGOR, J. H.—"The Spermatogenesis of Amphiuma," 'Journ. Morph.,' xv, Suppl., 1899.

Mann, G.—'Physiological Histology,' Oxford, 1902.

Mark, E. L.—"Maturation, Fecundation, and Segmentation of Limax campestris," 'Bull. Harvard Mus.,' vi, 1881.

Mead, A. D.—"Some Observations on Maturation and Fecundation in Chætopterus pergamentaceus," 'Journ. Morph.,' x, 1895.

Mead, A. D.—"The Origin of the Egg-centrosomes," 'Journ. Morph.," xii, 1897.

Mead, A. D.—"The Origin and Behaviour of the Centrosomes in the Annelid Egg," 'Journ. Morph.,' xiv, 1898.

Meves, F.—"Ueber die Entwickelung der männlichen Geschlechtszellen von Salamandra," 'A. m. A.,' xlviii, 1896.

Meves, F.—"Ueber Struktur und Histiogenese der Samenfäden von Salamandra," 'A. m. A.,' l, 1897.

Meves, F.—"Ueber Struktur und Histogenesis der Samenfäden des Meerschweincheus," 'A. m. A.,' liv, 1899.

Meves, F.—"Zelltheilung," 'Anat. Hefte qte. Abt.,' vi, 1896, and viii, 1898.

Michaelis, L.—"Die Befruchtung des Tritoneneies," 'A. m. A.,' xlviii, 1897.

Morgan, T. H.—"The Production of Artificial Astrospheres," 'Arch. f. Ent.-mech.,' iii, 1896.

Nicolas, A.—"Recherches sur l'Embryologie des Reptiles, Contribution à l'étude de la fécondation chez l'Orvet," 'Arch. d'Anat. Micr.,' iii, 1899—1900.

Oppel, A.—"Die Befruchtung des Reptilieneies,' 'A. m. A.,' xxxix, 1892.

Platner, G.—"Ueber die Befruchtung bei Arion empiricorum," 'A. m. A.,' xxvii, 1886.

Roux, W.—"Die Bestimmung der Medianebeue des Froschembryo durch die Copulationsrichtung des Eikernes und des Spermakernes," 'Ges. Werke,' xxi.

Rückert, J.—"Zur Befruchtung des Selachiereies," 'Anat. Anz.,' vi, 1891.

Rückert, J.—"Zur Entwickelungsgeschichte des Ovarialeies bei Selachiern," 'Anat. Anz.,' vii, 1892.

Rückert, J.—"Zur Eireifung bei Copepoden," 'Anat. Hefte,' iv, 1894.

Rückert, J.—"Zur Befruchtung von Cyclops strenuus," 'Anat. Anz.,' x, 1895.

Rückert, J.—"Die erste Entwickelung des Eies der Elasmobranchier," Festschr. f. C. von Kupffer, Jena, 1899.

Schaudinn, F.—"Ueber das Centralkorn der Heliozoen.," 'Verhandl. Deutsch. Zool. Gesellsch.,' 1896.

Schulze, O.—"Untersuchungen über die Reifrung und Befruchtung des Amphibieneies," 'Z. w. Z.,' xlv, 1887.

SOBOTTA, J.—"Die Befruchtung und Furchung des Eies der Maus," 'A. m. A.,' xlv, 1895.

SOBOTTA, J.—"Die Reifung und Befruchtung des Eies von Amphioxus lanceolatus," 'A. m. A.,' l, 1897.

STAUFFACHER, H.—"Eibildung und Furchung bei Cyclas cornea," 'Jenaische Zeitschr.,' xxviii, 1894.

VAN DER STRICHT, O.—"Contribution à l'étude de la Sphère attractive," 'Bull. Ac. Roy. Belg.,' iii, 23, 1892.

VAN DER STRICHT, O.—"La maturation et la fécondation de l'œuf d'Amphioxus lanceolatus," 'Arch. de Biol.,' xiv, 1896.

VAN DER STRICHT, O.—"La formation des deux globules polaires et l'apparition des spermo-centres dans l'œuf de Thysanozoon Brocchii," 'Arch. de Biol.,' xv, 1898.

VEJDOVSKY, F.—"Entwickelungsgeschichtliche Untersuchungen," Prag., 1888—1892.

VEJDOVSKÝ, F., and MRÁZEK, A.—"Umbildung der Cytoplasma während der Befruchtung und Zellteilung," 'A. m. A.,' lxii, 1903.

WHEELER, W. M.—"The Behaviour of the Centrosomes in the Fertilised Egg of Myzostoma glabrum," 'Journ. Morph.,' x, 1895.

WILSON, E. B., and MATTHEWS, A. P.—"Maturation, Fertilisation, and Polarity in the Echinoderm Egg. New light on the 'Quadrille of the Centers,'" 'Journ. Morph.,' x, 1895.

WILSON, E. B.—"Archoplasm, Centrosome and Chromatin in the Sea-urchin Egg," 'Journ. Morph.,' xi, 1895.

WILSON, E. B.—"Atlas of the Fertilisation and Karyokinesis of the Ovum," New York, 1895.

WILSON, E. B.—"The Cell in Development and Inheritance," New York, 1902.

WILSON, E. B.—"Experimental Studies in Cytology. I. Artificial parthenogenesis in Sea-urchin Eggs," 'Arch. f. Entw. mech.,' xii, 1901.

ZIEGLER, H. E.—"Experimentelle Studien über die Zelltheilung," I. 'Arch. f. Ent. mech.,' vi, 1898.

EXPLANATION OF PLATES 29—33,

Illustrating Mr. J. W. Jenkinson's paper on "Observations on the Maturation and Fertilisation of the Egg of the Axolotl."

All the figures were drawn with the aid of Zeiss' camera lucida; comp. oc. 6, achr. obj. 2mm. magn. 750×.

Figs. 1—14. Maturation.

Fig. 1.—Metaphase of first polar spindle. At the outer end may be seen some astral rays. The inner end is bi-polar.

Fig. 2.—Telophase of first polar spindle. The chromosomes have united into an annular skein. The surface of the egg is raised up into a flat disc; the beginning of the first polar body.

Fig. 3.—Formation of the first polar body. This is still united to the egg by a narrow stalk in which Zwischen-körper are seen. The chromosomes are again distinct.

Fig. 4.—The first polar body is completely separated. The chromosomes in it have divided longitudinally, the chromosomes of each pair being united by their apices. In the egg the chromosomes have also divided, and lie in a tangentially elongated striated clear area, the first sign of the second polar spindle.

Fig. 5.—Metaphase of the second polar spindle from a freshly laid egg preserved in aceto-corrosive. Note the fibre-bundles attached to the apices of the chromosomes. The latter are paired and lie in the equator. The outer spindle pole is slightly depressed.

Fig. 5 *a*.—The same, cut across. The apices of the chromosomes point towards the spindle axis.

Fig. 6 *a*.—The same, but form an oviducal egg preserved in picro-acetic. The chromosomes, scattered irregularly over the spindles, are beginning to diverge by their apices. Note the "Zugfasern" and the "Verbindungsfäden." The outer spindle pole projects above the surface.

Fig. 6 *b*.—The same as the last, but preserved in chromo-acetic.

Fig. 7 *a*.—Late anaphase of the second polar spindle. There are no "Zugfasern" to be seen. Note the outer fibres diverging into the equatorial plane.

Fig. 7 *b*.—The same as last, but a little later; the first stage in the formation of the second polar body.

Fig. 8.—The second polar body completely formed, but not yet quite constricted off. Note the protrusion of the vitelline membrane. The chromosomes in both polar body and egg converge by their apices; in the latter they lie in a clear area.

Fig. 9.—First polar body, cut equatorially. Notice vacuolated cytoplasm, agglomerated yolk-granules, pigment and cruciform jagged chromosomes.

Fig. 10.—First polar body with nucleus partially reconstituted. The chromosomes, though still distinct, lie in an oval area. This, however, may possibly be one of the products of division of the first polar body (see text).

Figs. 11—14.—Second polar body showing the reconstruction of the nucleus. Figs. 11, 12 and 14 are cut equatorially. Notice vacuolated cytoplasm, pigment and clumps of yolk-granules. In Fig. 11 there are vacuoles round the chromosomes. In Fig. 12 these vacuoles have united into one oval nuclear vacuole, the wall of which forms the nuclear membrane; the chromosomes are still distinct. In Fig. 13 the chromosomes are still distinct, but are sending out processes to one another and to the wall, while in Fig. 14 they have given rise to a very coarse reticulum.

Figs. 15—41. Fertilisation.

Fig. 15.—The spermatozoon with head, middle-piece and tail lying in a clear area, slightly pigmented, but devoid of yolk-granules, the sperm-sphere. The tail (on the left) is pointing towards the sperm-path.

Fig. 16.—A little later. The sperm-head has shortened and thickened; the tail is seen to the right. The middle-piece has vanished. Instead, the centre of the clear area is now occupied by a vacuolated pigment-free mass. From this start the radiations of the sperm-aster which have meanwhile been developed.

Fig. 17.—A little earlier than the last. The central mass is finely radiate, and in it is a small irregular vacuolated body which may be middle-piece or perhaps tail. The rest of the sperm-head is in the next section.

Fig. 18.—A little later than Fig. 16. The sperm-head has become shorter and thicker still; it is obtusely conical. Its vacuolation has increased.

Fig. 19.—An accessory sperm-nucleus with centrosome. The nucleus contains large plasmosomes staining black with iron-hæmatoxylin, and minutely divided granules of chromatin; these stain faintly. There is an achromatic reticulum. The centrosome lies in front of (right-hand side in the figure) the nucleus; between it and the nucleus are fine parallel "spindle" fibres. It is granular. Large vacuoles are developing in the centre of the sperm-aster.

Fig. 20.—Sperm-nucleus in an earlier stage, coarsely reticular (the section does not pass through the middle of the nucleus, the full length of which has

not therefore been shown). Centrosome about to divide. Note the cloud of pigment. The sperm-path is on the left side.

Fig. 21.—Centrosome elongated. The rest as in Fig. 19.

Fig. 22.—The daughter centrosomes have moved apart. The (accessory) sperm-nucleus is coarsely reticular, and the nuclear membrane is hard to see on the right-hand side. The large size of the yolk-granules is due to the sperm having entered below the equator. Depigmented preparation; originally like Fig. 23.

Fig. 23.—In the (accessory) sperm-nucleus the chromatic portion is crowded into the centre. Towards the cloud of pigment which obscures the centrosomes the nuclear membrane is very much weakened. This sperm also has entered below the equator.

Fig. 24.—Origin of the centrosome from the (accessory) sperm-nucleus. Note the closeness of the centrosome to the nucleus, the absence of a membrane here, and the pigmented processes running up into the nuclear cavity.

Fig. 25.—Exactly as the last, but nucleus and centrosomes are cut consecutively. Four consecutive sections; *a* is the topmost, *d* at the bottom of the series, and the pigment in *d* is over the centrosome. In the nucleus the chromatin is crowded together centrally.

Fig. 26.—Sperm-nucleus and centrosome. *a*. The centrosome, granular. *b*. The nucleus, very coarsely reticular, and consequently in an earlier stage than in Figs. 19—25.

Fig. 27.—Annular dividing centrosome. Division later than usual, the pronuclei having met.

Fig. 28.—Formation of the female pronucleus. *a*. Membrane formed, but chromosomes still distinct. *b*. Chromosomes breaking up. *c*. Chromatin coarsely granular; a chromatic reticulum clearly visible. *d*, *e*. Chromatin minutely subdivided, pronucleus enlarged and lobed. In *d* a few vacuoles between the pronucleus and the yolk-granules.

Fig. 29.—The pronuclei have met. The male pronucleus is on the left; in it the chromatin is aggregated centrally. The centrosomes have moved apart, in a direction at right angles to the line joining the pronuclei. Note the pigment, and the vacuoles of the sperm-aster.

Fig. 30.—The same as the last, but only one pronucleus is shown. Note the fine parallel "spindle" fibres between it and the centrosomes. Note also the enormous central vacuoles of the sperm-aster with the remains of the separating lamellæ, and the astral rays passing out between the yolk-granules.

Fig. 31.—Early stage in the formation of the fertilisation spindle. Notice the fine parallel spindle-fibres between the centrosomes and the pronuclei; and the large terminal vacuoles of the elongated sperm-sphere. The plasmosomes are stained black with iron-hæmatoxylin.

Fig. 32.—Later. The terminal vacuoles are reduced. The pronuclei are elongated parallel to the spindle axis. A pigmented cord still connects the centrosomes. Plasmosomes as in the last.

Fig. 33.—Later still. The centrosomes are much enlarged, and the terminal vacuoles have disappeared. From each centrosome pass out a number of fine "inner" astral rays (see text). Note the round vacuoles at the equator.

Fig. 34.—Only one pronucleus is shown; the chromosomes are forming in it. The achromatic reticulum is coarse, and bears granular thickenings. The spindle is much longer, the centrosomes smaller and reticular (aceto-corrosive preparation), and the inner astral rays exceedingly fine.

Fig. 35.—Resting nucleus of one of the first two blastomeres; in it are seen plasmosomes, finely divided chromatic granules, and an achromatic reticulum. On its polar—the right—side is a depression, and on the same side two small centrosomes. It lies in a clear, much vacuolated area.

Fig. 35 *a*.—Division of the centrosomes in the anaphase of the fertilisation-spindle. The centrosomes are flattened against one another; each is lobed and contains a centriole. Chromo-acetic preparation.

Fig. 36.—Early stage in the formation of the chromosomes by linear aggregation of granules. In the female pronucleus (on the left) a plasmosome is still visible. In the male pronucleus there is a very coarse granular network of chromatin crowded together in the centre of the pronucleus. In both pronuclei the achromatic reticulum is coarse.

Fig. 37.—Transverse section of the fertilisation spindle in early metaphase showing two distinct sets of chromosomes.

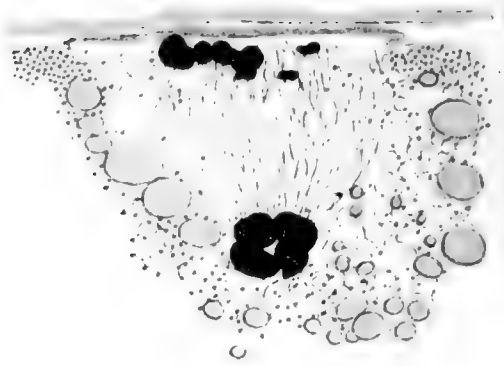
Fig. 38.—Formation of the equatorial portion of the spindle from the achromatic reticulum of (one of the) pronuclei. The continuity of the extra- and intra-nuclear fibres through the openings in the membrane of the upper pronucleus is readily seen. Centrospheres and centrosomes as in the next figure. Pronuclei as in Fig. 34.

Fig. 39.—Early metaphase. Aceto-corrosive preparation. The inner rays have undergone reticular degeneration and now form the centrospheres. In each centrosphere is an ill-defined reticular centrosome. The spindle-fibres are undulating, united by anastomoses, and pass continuously from pole to pole. Outside the spindle is a mantle of equatorial astral rays; these are closely pressed together and pigmented. The chromosomes lie unevenly in the equatorial plane.

Fig. 40.—Metaphase. Aceto-chromic preparation. The chromosomes are split, lying in the equator. To each pair of chromosomes is attached a pair of special fibre-bundles ("Zugfasern"). The centrospheres are reticular and contain each a homogeneous lobed centrosome; inside each of these the centriole has divided.

Fig. 41.—Anaphase. Aceto-chromic preparation. Centrospheres and centrosomes as in the last, except that the centriole is undivided. The fibre-bundles attached to the ends of the chromosomes are pulling the latter apart; where the point of attachment is subterminal the end of the chromosome is clearly hooked. The equator is occupied by an achromatic plate, and the peripheral spindle-fibres clearly turn outwards to become parallel with the plane of the equator.

Fig 1



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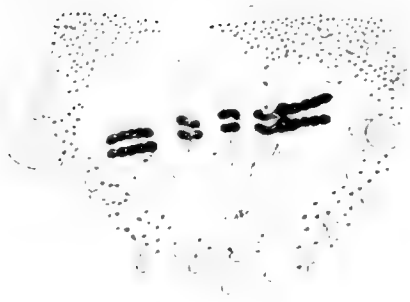


Fig. 5.

Fig. 6a.

Fig. 9.

Fig. 6b

Fig. 10.

Fig. 5a.

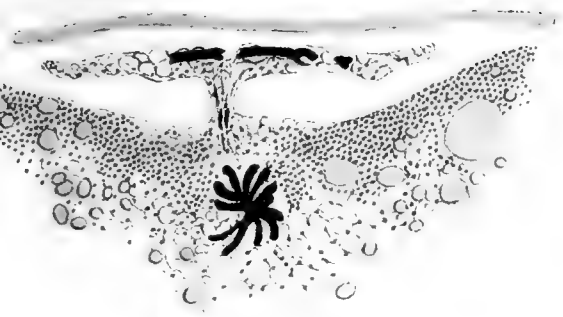


Fig. 3.

Fig. 4.

Fig. 7b.

Fig. 7a.

Fig. 8

Fig. 12.

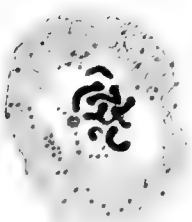
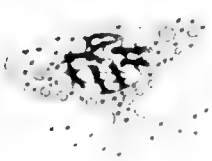


Fig. 11



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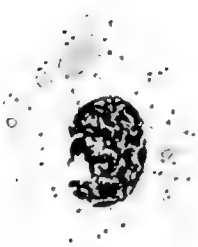

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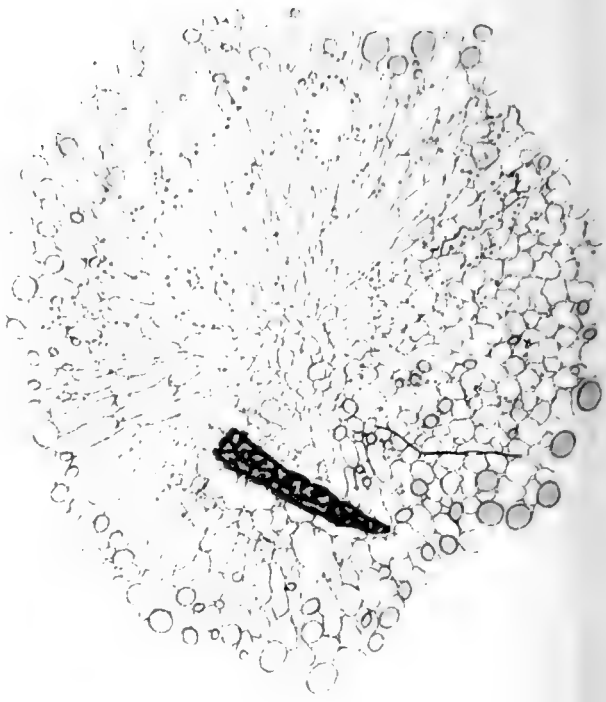
Fig. 15.



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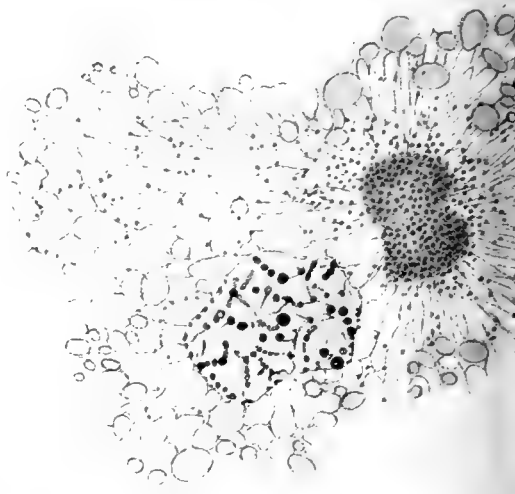

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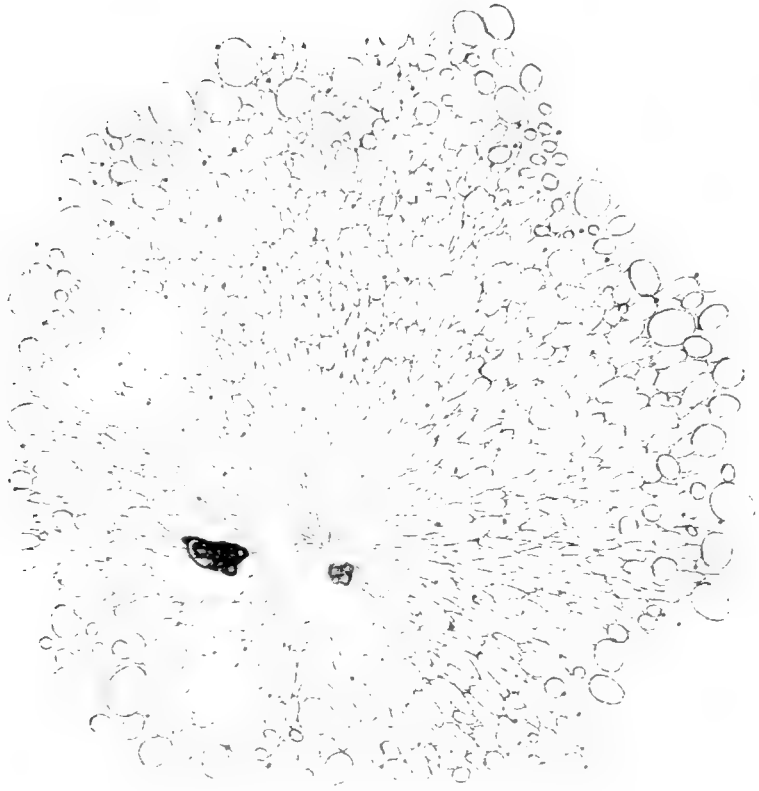

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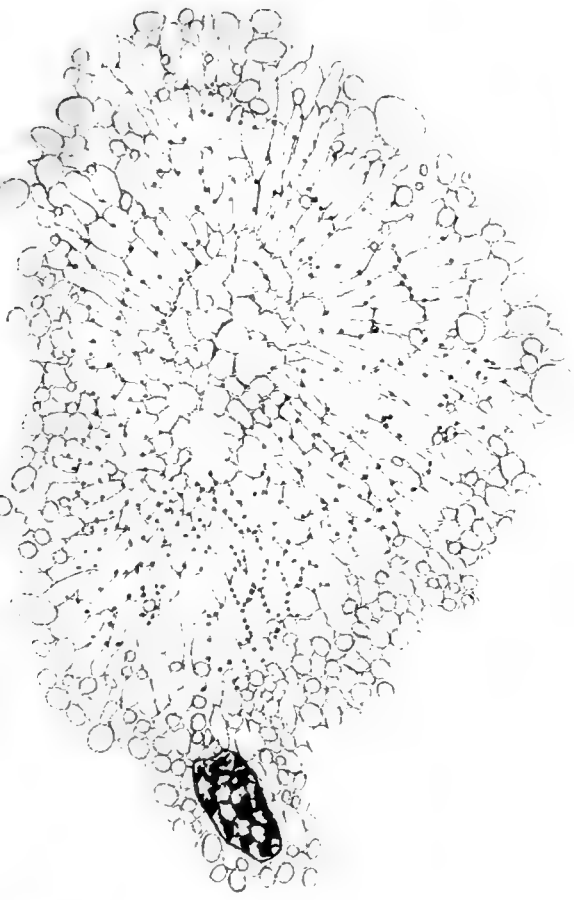

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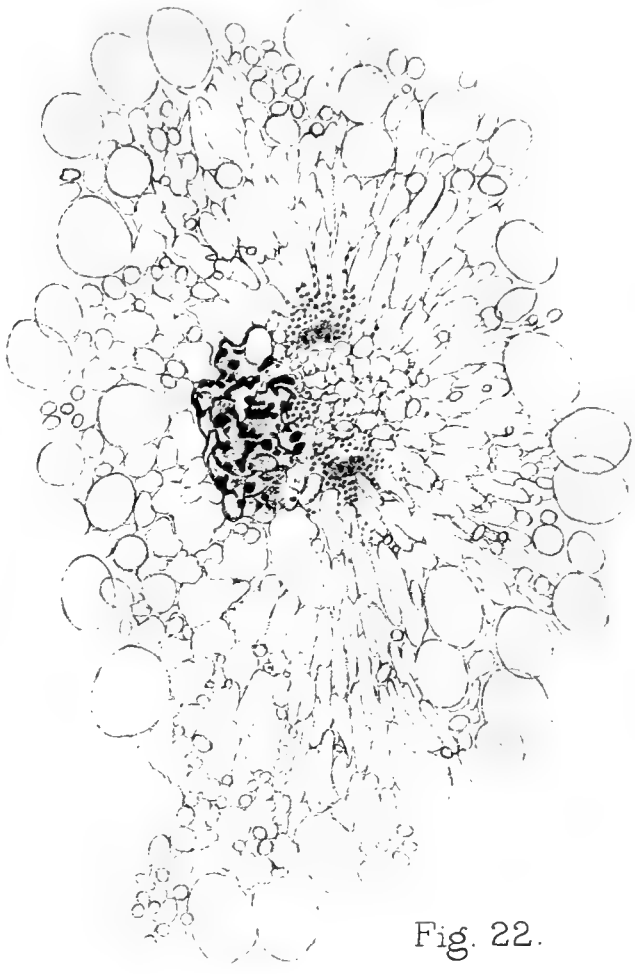

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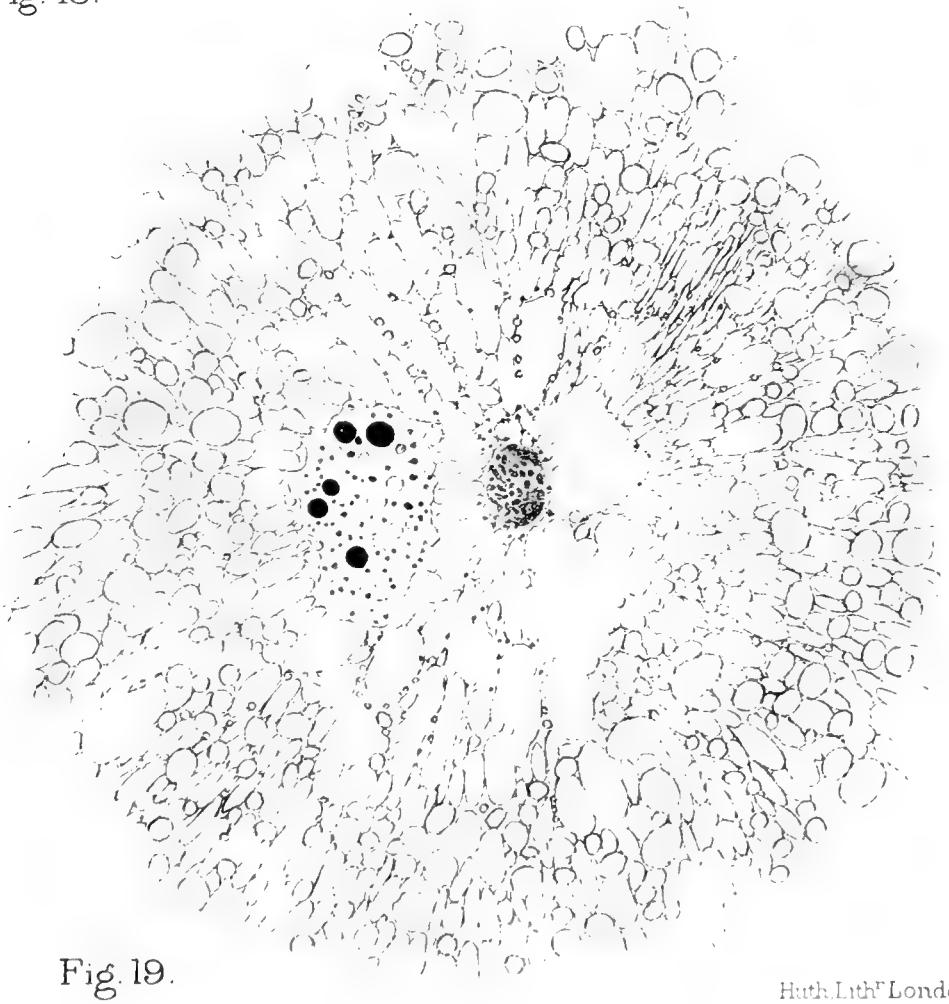

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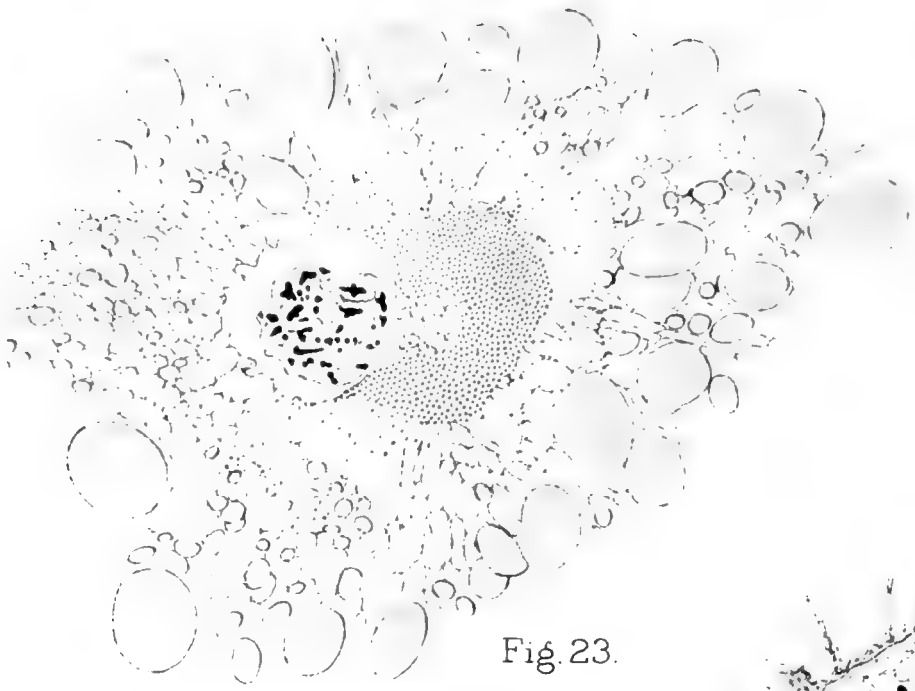
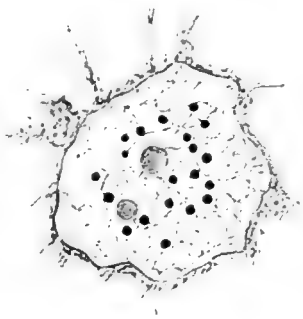


Fig. 23.

Fig. 27.



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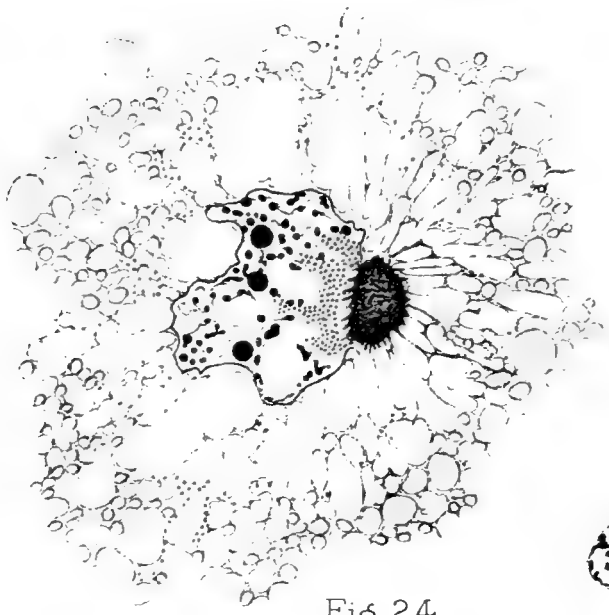

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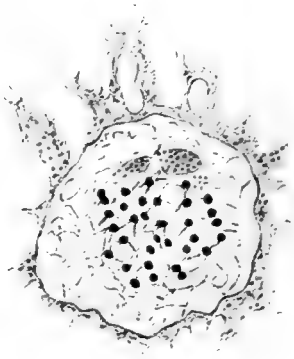

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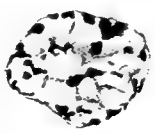

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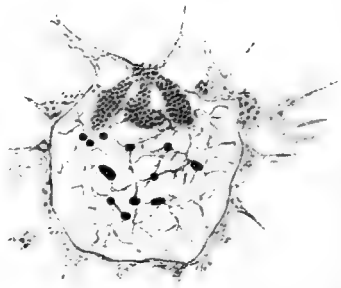

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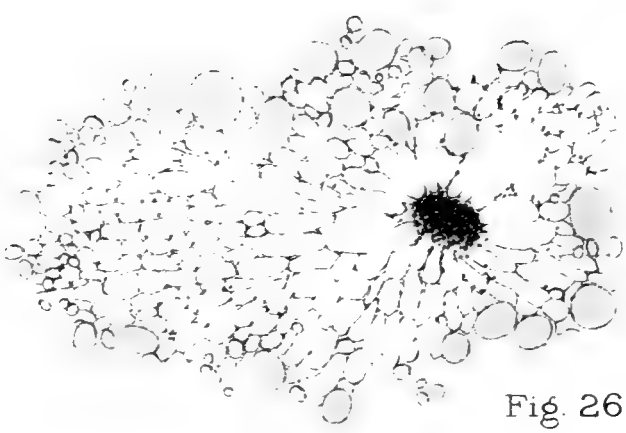


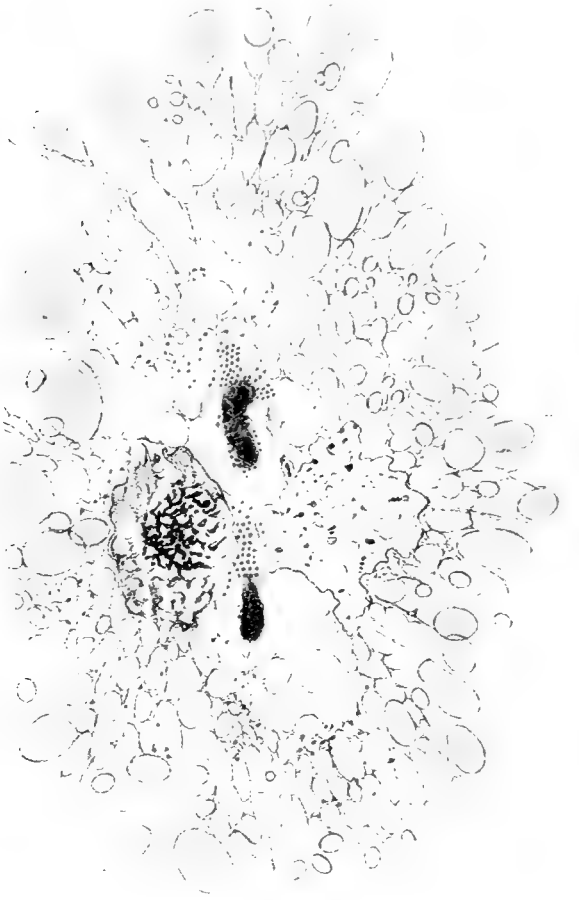
Fig. 26 a.

Fig. 25 d.

Fig. 28 a.

Fig. 28 b.

Fig. 28 c



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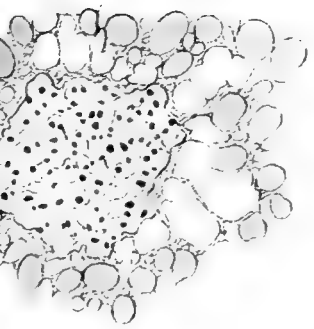

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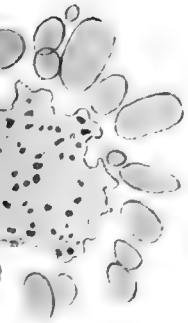

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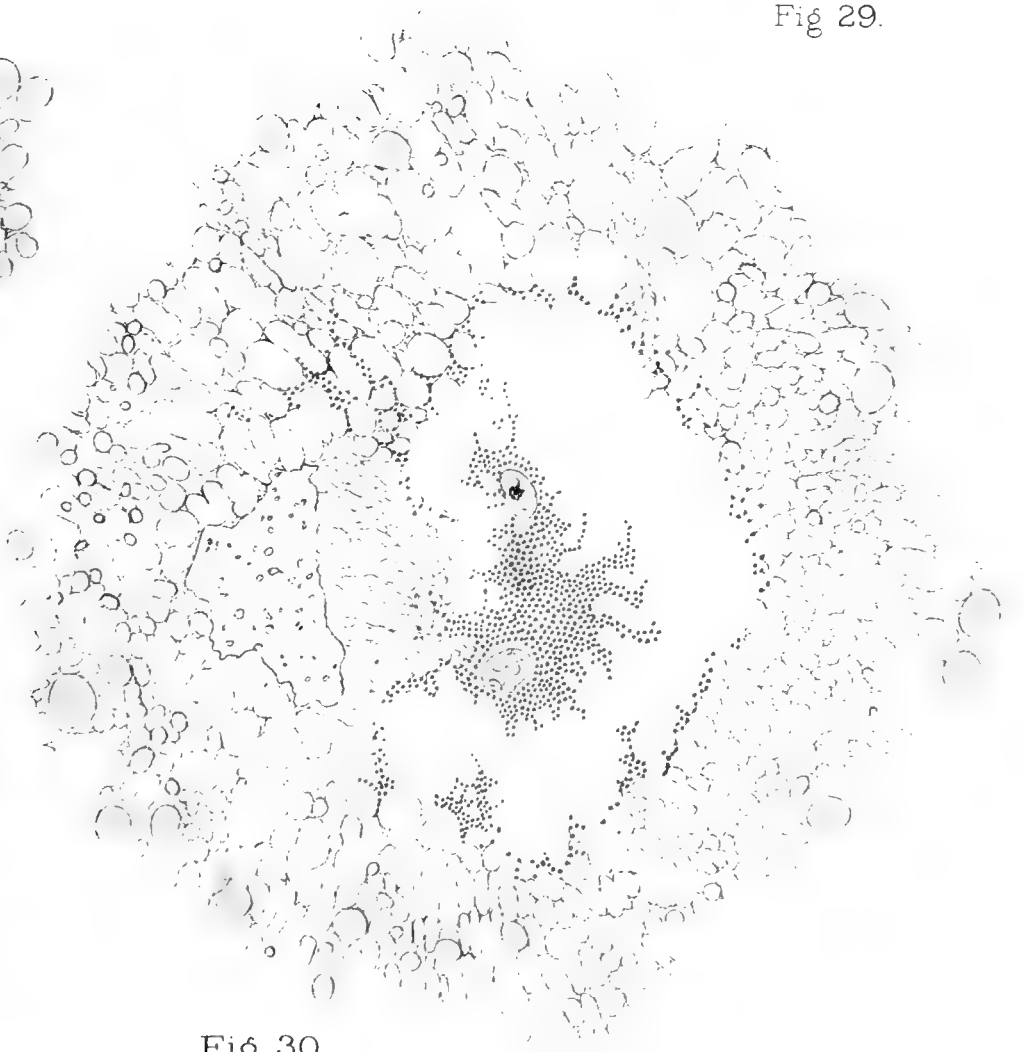

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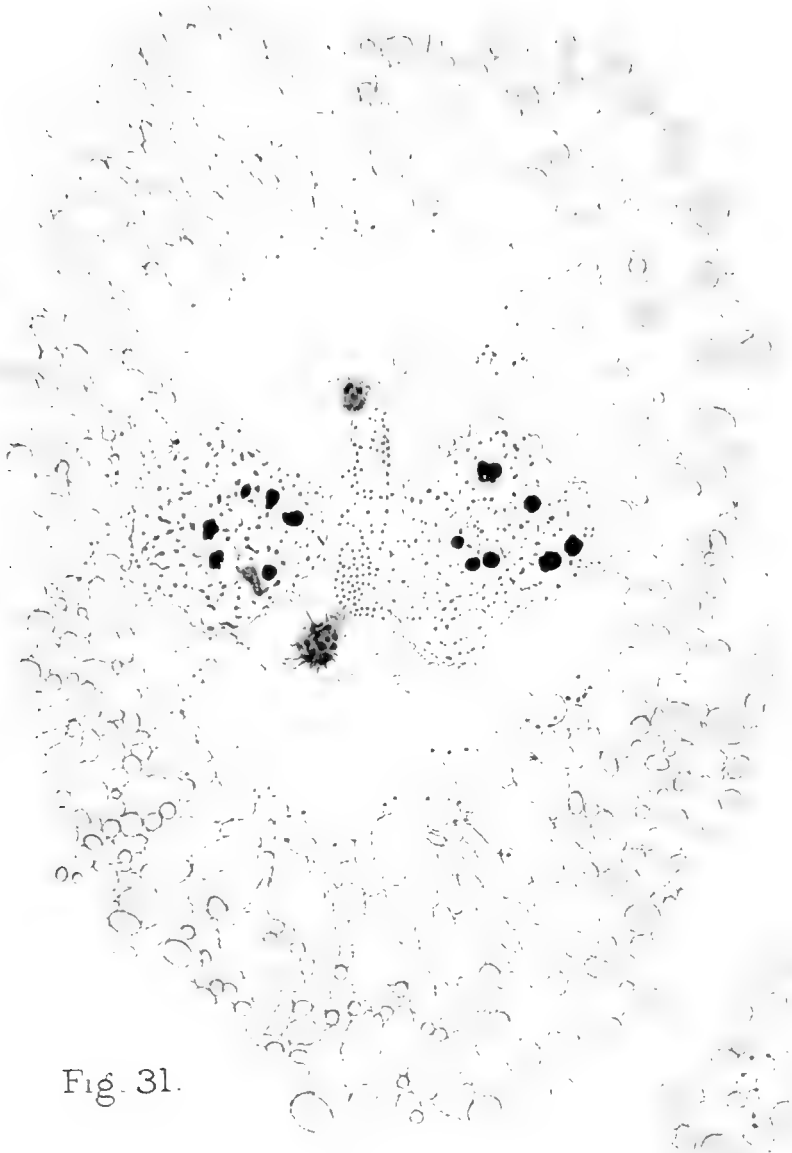
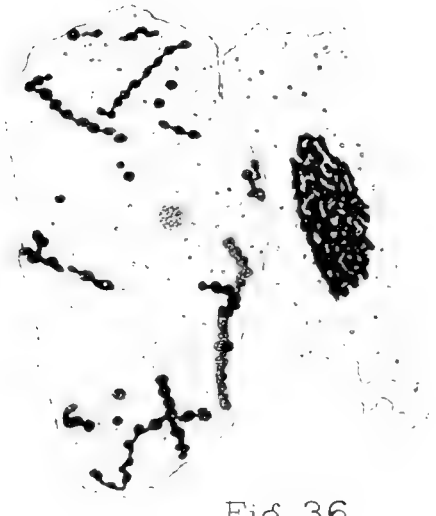


Fig. 31.

Fig. 37.



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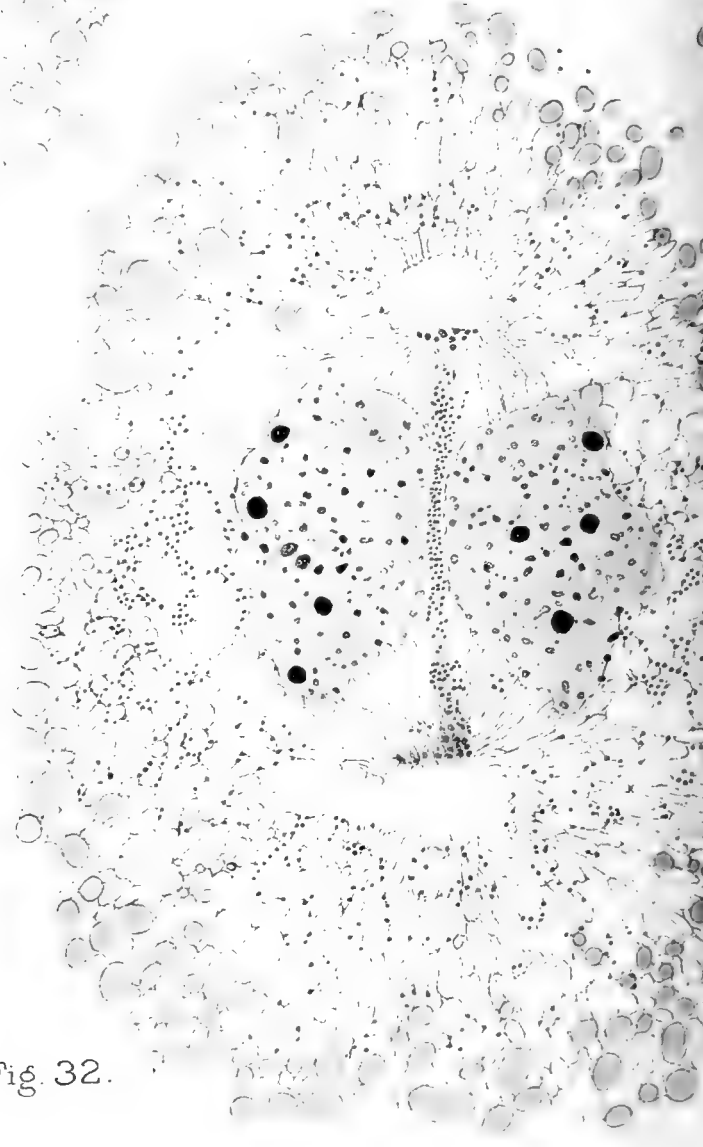


Fig. 32.

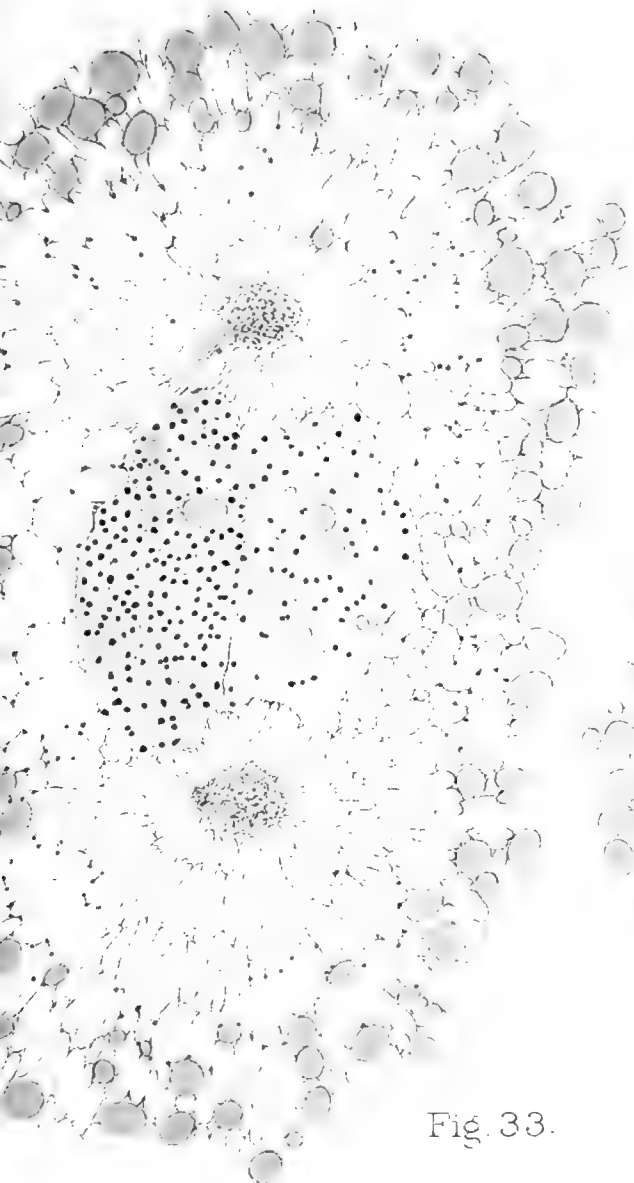


Fig. 33.

Fig. 34.

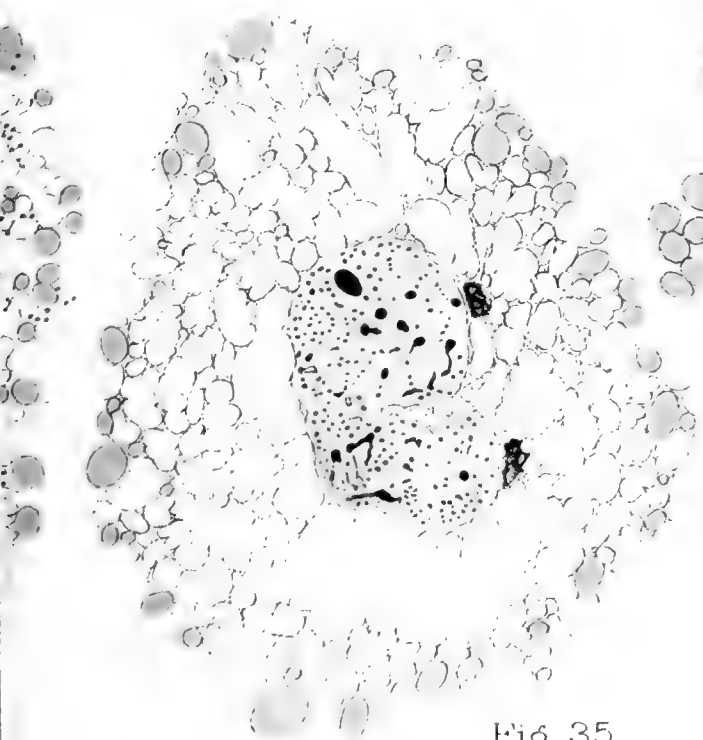


Fig. 35.

Fig. 35a.

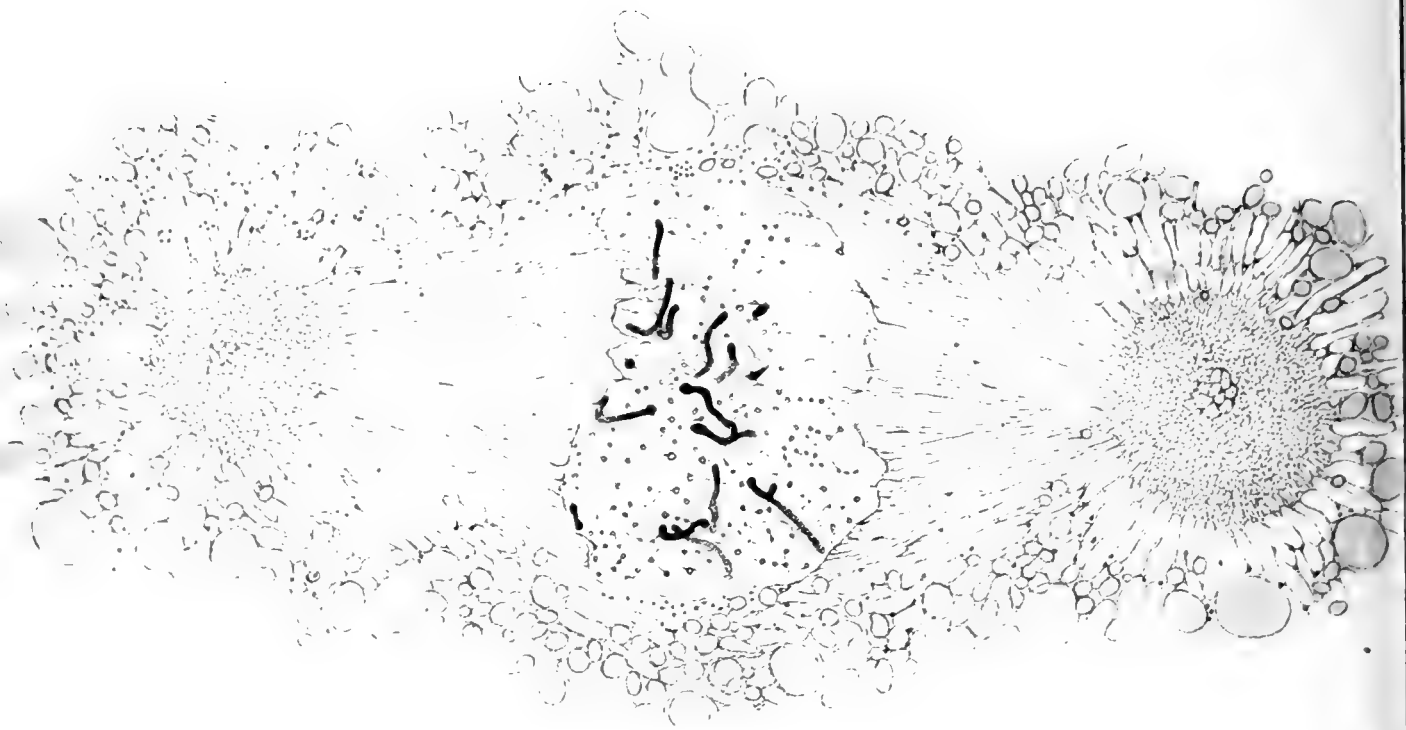


Fig. 38.

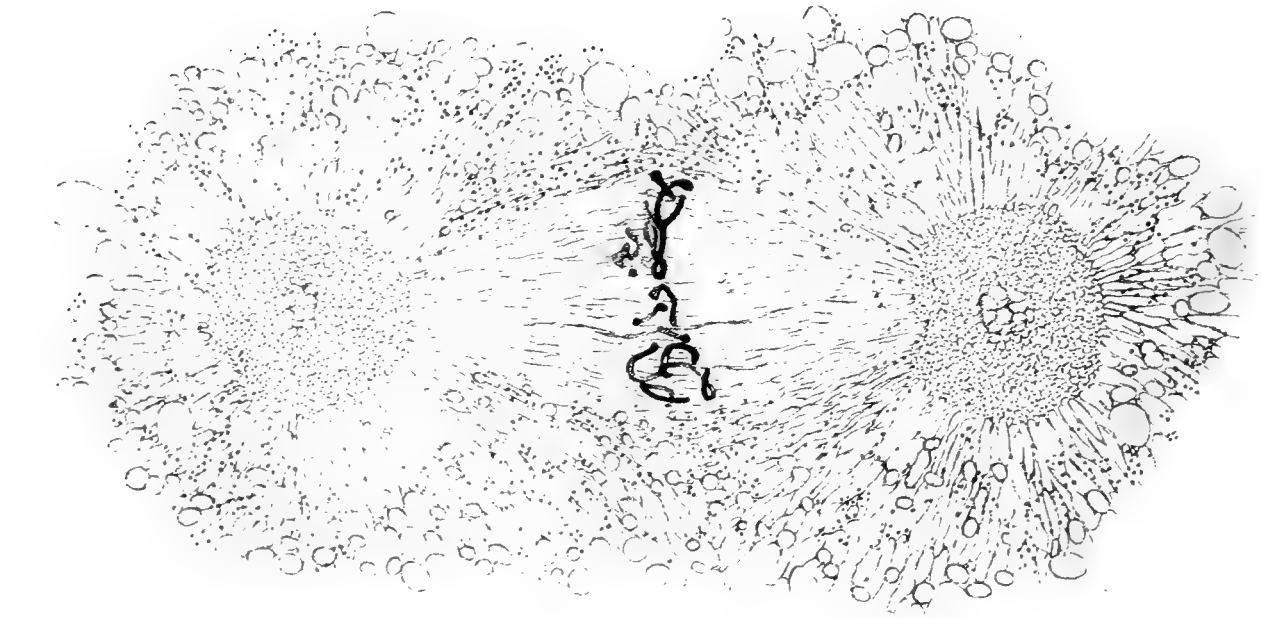


Fig. 39.

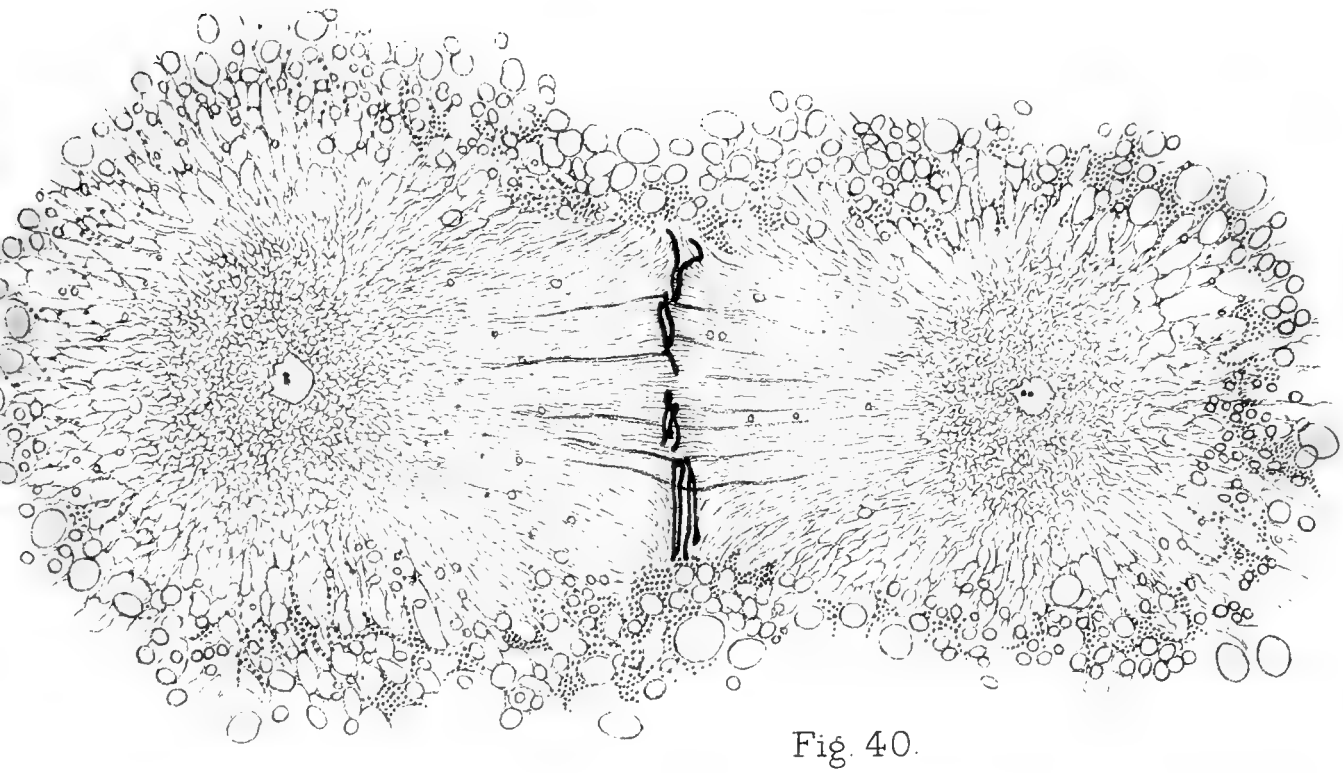


Fig. 40.

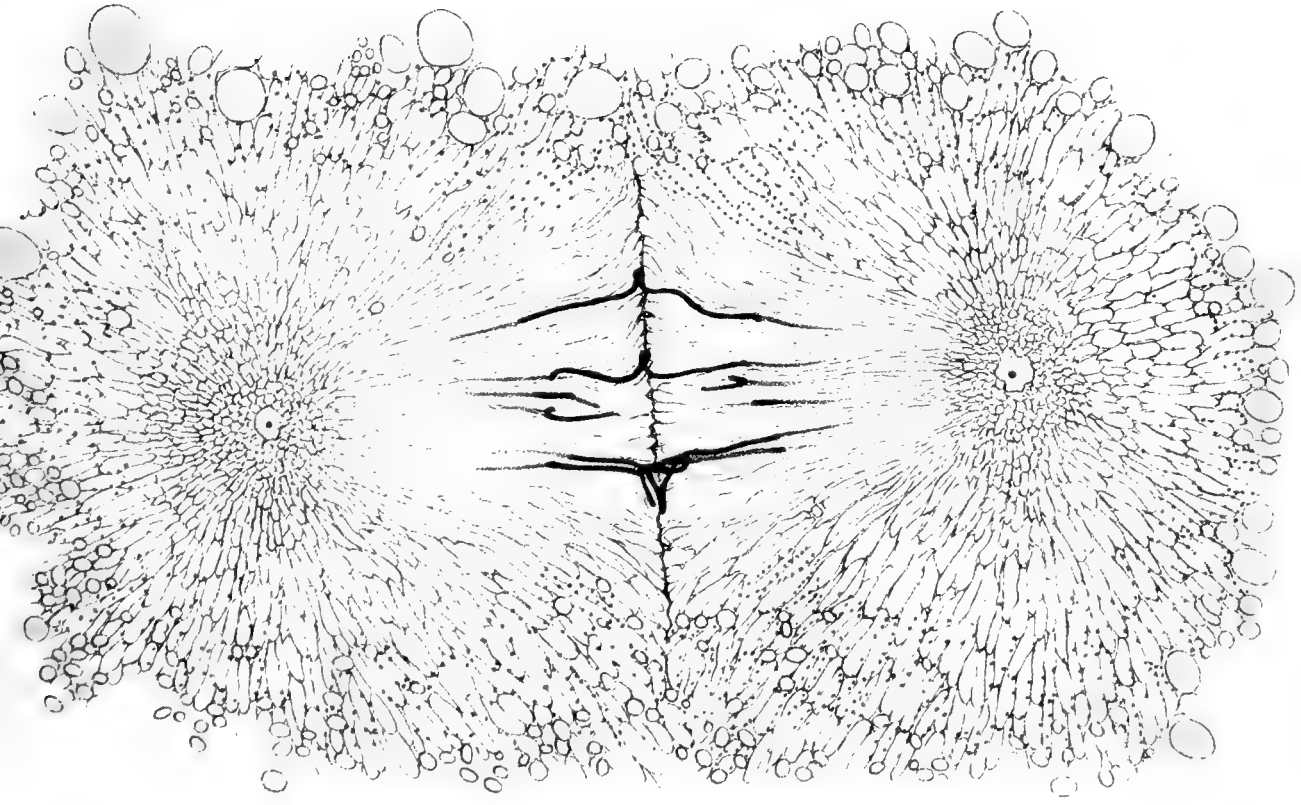


Fig. 41.

Huth, Lith. London.

Notes on the Anatomy of Gazelletta.

By

G. Herbert Fowler, B.A., Ph.D., F.Z.S., F.L.S.

In a recent paper I described, as completely as the state of preservation of the material would permit, the anatomy of Planktonetta atlantica, Borgert,[1] a remarkable type of Phæodarian Radiolarian. Associated with this species were some specimens of Gazelletta, probably G. fragilis, named by Dr. Borgert from broken material obtained by the National.[2] I am obliged to him for permission to publish a short note upon the main points in which it differs from Planktonetta. As, however, this organism is even more fragile, and therefore worse preserved than the former, and as my specimens were fewer in number, the only excuse for so incomplete an account lies in the structural novelty of the interesting family (Medusettida) to which it belongs.

It seems probable that my collection included at least two species. Of five specimens cut for sections, one had a very thick body-wall, the others only a comparatively thin wall; of the loose bodies found in the material, most are of the thick-walled type. The anatomical relations seem, however, to be the same in both cases. Fig. 2 is taken from a specimen with a thin capsule; Fig. 1 from one with a thick gelatinous wall; the latter type appears to have a special membrane lining the interior, of which no trace could be detected in the former.

For descriptive purposes, and until a special terminology

[1] 'Quart., Journ. Micr. Sci.,' xlvii, 133.

[2] 'Zool. Jahrbücher (Abth. Syst. u. s. w.),' xvi, 570.

is called for, Gazelletta may be divided into the body (? = central capsule) and head (="shell-mouth" and arms), the intra-capsular protoplasm and nucleus lying in the body, the extra-capsular protoplasm and phæodium in the head. The body is nearly spherical or ovoid. The body-wall stains deeply in hæmatoxylin, is soft and elastic, and shrivels very greatly in preparation for sections. It seems to me to be homologous with the central capsule rather than with the shell of Planktonetta, because it is the only recognisable membrane in the position of a central capsule, and it shows no sign of being continuous with the shell-mouth, which is

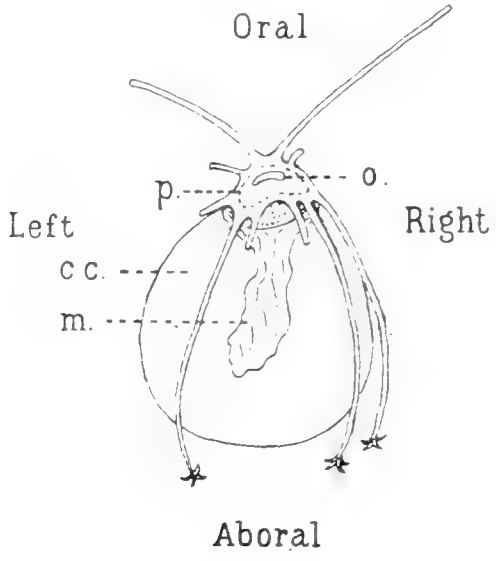


FIG. 1.—Specimen with thick body-wall, and with ten arms, most of which have been broken; all except the most anterior pair should lie more or less by the side of the body. Drawn from the "posterior" side; the terminal spines of the arms alone have been drawn. *c. c.* Body (central capsule?); *m.* its internal lining membrane; *o.* alleged opening of the shell-mouth; *p.* row of pores.

undoubtedly skeletal. It is continued as a very thin membrane over the "oral" surface of the intra-capsular protoplasm, where it is perforated by the suspensory processes and by the bundle of communicating tubes, as in Planktonetta. These processes and tubes are the only apparent means by which the body is attached to the remainder of the organism, but I dare not state positively

that the body-wall is not also continuous with the edge of the diaphragm, a condition which seemed to be probable in Planktonetta. The attachment being so slight, one naturally finds numerous separate heads and bodies, but only a few specimens in which they are still united; the separation takes place between diaphragm and central capsule. If one has

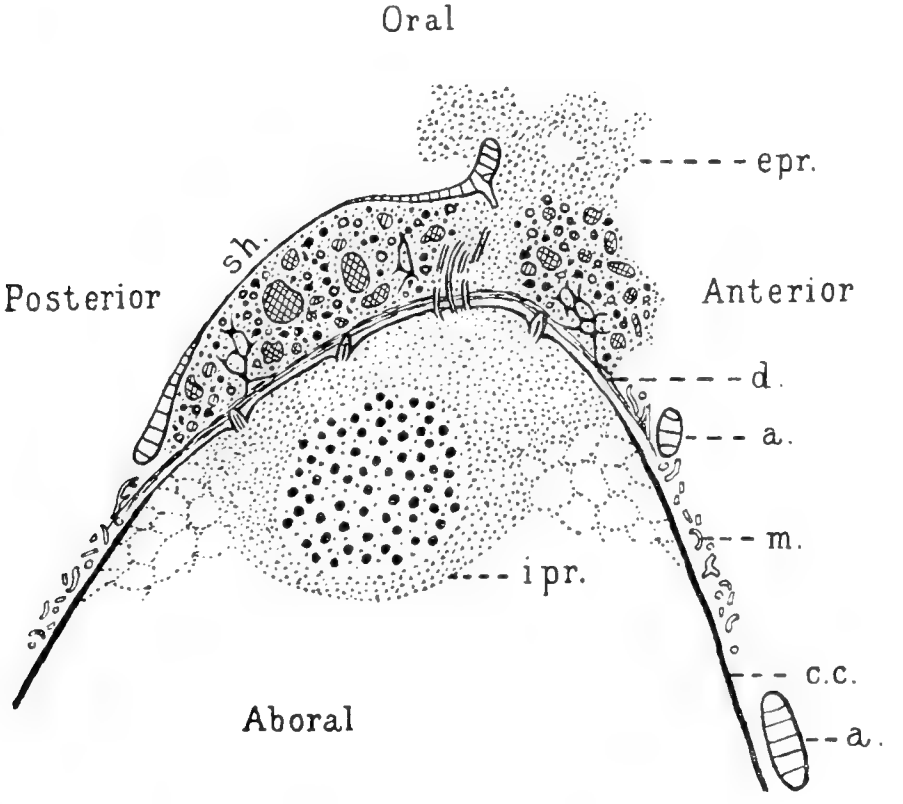


FIG. 2.—Diagrammatic section of the central portion of a specimen with thin body-wall, founded on camera drawings. *a.* Oblique sections of arms; *c. c.* body-wall (central capsule?) perforated above by suspensory processes and by the bundle of communicating tubes between extra- and intra-capsular protoplasm; *d.* diaphragm; *e. p. r.* extra-capsular protoplasm free from phæodial corpuscles, protruding from under the shell; centrally it shows portions of the tubes by which it communicates with the interior of the capsule; in the remainder of the extra-capsular protoplasm the phæodial corpuscles and portions of the skeletal meshwork are diagrammatically indicated; *i. p. r.* intra-capsular protoplasm containing the large nucleus; *m.* skeletal meshwork between the arms, which apparently serves for the attachment of the diaphragm; *sh.* shell.

either body or head alone before one, it is not possible to infer the existence of the other part. The intra-capsular

protoplasm is of the same character as in Planktonetta, but the suspensory processes are fewer and more slender. The shell-mouth (to use temporarily the same term as in Planktonetta) has been figured by Dr. Borgert (op. cit.); having only the head before him, he made the natural mistake of thinking that the larger opening was oral, the smaller (if it really exist) aboral; but the reverse is the case, and his figure is drawn from the "oral" aspect. I am not convinced that the smaller opening has a real existence, but I incline to think that in life it is occupied by a thin film of shell, which disappears in the process of cleaning. If present, it is certainly not the mouth, as will appear shortly.

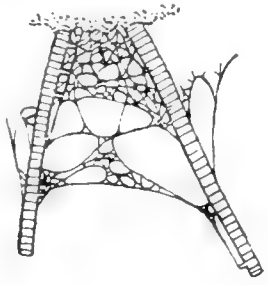


FIG. 3.—The central ends of two arms projecting out from under the protoplasm, showing the skeletal meshwork.

The large "aboral" opening is closed below by a fibrous diaphragm; the circumference of this is not inserted into pits of the shell-mouth, as in Planktonetta, but is apparently attached to, or continuous with, a skeletal meshwork developed between the aborally directed arms. Into this diaphragm are inserted the suspensory processes of the intracapsular protoplasm, and it is perforated by the communicating tubes.

The shell-mouth is slightly saddle shaped, the lappets of the saddle lying right and left of the organism, but its rim is raised a little anteriorly.[1]

The arms, according to Dr. Borgert, are 8—10 in number, in my cleaned specimens 10—13. The anterior pair are

[1] In Dr. Borgert's drawing the right side of the structure is lowest in the figure; the "anterior" edge is on the right of the figure.

directed away from the body, more or less in the long axis of the organism; most, if not all, of the rest lie at the sides of the body, directed aborally. Between these aboral arms is developed a skeletal meshwork (Fig. 3), serving for the attachment of the diaphragm, and to some extent protecting the body; it is borne on the spines of the arms, and lies between them and the body. The general relations of the shell-mouth are obvious in Figs. 1 and 2, and its finer structure has been adequately figured by Dr. Borgert.

The extra-capsular protoplasm is less voluminous than in Planktonetta; but is similarly divisible into (a) a highly vacuolated portion charged with phæodial corpuscles, lying mainly posteriorly and laterally, but also present anteriorly and (b) an anterior protoplasmic mass devoid of phæodium. This mass, which presumably marks the point of ingestion and egestion of food, does not approach the alleged smaller opening of the shell, but projects from under the raised anterior lip of the saddle-shaped shell-mouth. Through protoplasm and phæodium runs a fine skeletal meshwork, as in Planktonetta.

As regards the distribution of these two Medusettids, there can be no doubt that they were, at the date and place of capture (extending to nearly three weeks), purely confined to the upper Mesoplankton, with a centre of distribution at or somewhat below the 100-fathom horizon. They were captured as shown in the table.

Open nets, towed at the depth indicated for half to one hour, then hauled to surface:

In	0	hauls out of	25	= 0 per cent. at	0	fathoms.	
,,	0	,,	12	= 0 ,,	25	,,	
,,	2	,,	13	= 15 ,,	50	,,	
,,	3	,,	11	= 27 ,,	75	,,	
,,	17	,,	22	= 77 ,,	100	,,	

Mesoplankton closing net:

In	4	hauls out of	7	= 77 per cent. at	200	to	100	fathoms.
,,	1	,,	3	= 33 ,,	250	,,	150	,,
,,	0	,,	3	= 0 ,,	300	,,	200	,,

They occurred in no haul which closed at a greater depth than 200 fathoms.

It will have been apparent that the terms of orientation used in describing Planktonetta, however suitable there, are really inapplicable to Gazelletta; nevertheless they have been used in these notes in order to avoid unnecessary multiplication of temporary terms. Although it would have been easy to coin pseudo-classicisms for the various parts, they would not fit the anatomy of the next Medusettid described, should it differ as much from these two as they do from one another. What really is the shell-mouth in Planktonetta, i. e. a ring round the point of ingestion, is in Gazelletta a shell-cap over the extra-capsular protoplasm; the body-shell of Planktonetta is (apparently) not represented in Gazelletta; and the terms "oral," "aboral," "anterior," "posterior," will probably have to be altered as our knowledge of the family increases. The fixed point in both seems to be the bundle of connecting tubes. At present it appears likely that the intrinsic shell is what I have termed the shell-mouth; this may cover (Gazelletta) or encircle (Planktonetta) the point of ingestion; it may also be continued aborally so as to surround the central capsule (Planktonetta). The float of Planktonetta is doubtless a subsidiary structure, as it is only attached by the spines and meshwork to the central shell.

On the Maiotic Phase (Reduction Divisions) in Animals and Plants.[1]

By

J. Bretland Farmer, D.Sc., F.R.S.,

AND

J. E. S. Moore, A.R.C.S., F.L.S.

With Plates 34—41.

Introduction.

We think it desirable, in the interests of clearness, to explain the meaning of the nomenclature that is employed in this memoir in connection with the "reduction" divisions.

We propose to apply the terms Maiosis or Maiotic phase[2] to cover the whole series of nuclear changes included in the two divisions that were designated as Heterotype and Homotype by Flemming.

Our reason for introducing this terminology is in order to emphasise the fact that these two mitoses invariably constitute a perfectly definite and recognisable phase, and one which is normally intercalated in the cellular life-cycle of all metazoa and metaphyta in which the sexual union of gametes takes place.

The actual point in the life-history at which the maiotic phase may occur is not identical in every organism, and it is

[1] This paper contains the evidence on which our preliminary communication to the Royal Society in May, 1903, was based. Its earlier publication has been delayed by the pressure of other work.

[2] *μείωσις*, reduction; *μειωτικός*, that which is reduced.

only the essential details within the phase itself that admit of complete comparison in the case of some of the more widely sundered groups—such, for example, as animals and plants respectively.

On the one hand, in the metazoa the divisions included in the maiotic phase invariably lead directly to the formation of the sexual cells. In plants, on the other hand, not only is the position of the phase far more variable, but it never culminates, so far as is known, directly in the production of sexual cells. The latter are only formed after a greater or less number of intervening (post-maiotic) divisions have been passed through.

It is evident, then, that we may group the cells that are produced in the life cycle of an animal or plant into three categories, viz. Premaiotic, Maiotic, and Post-Maiotic respectively. The convenience of this classification will at once be obvious. Thus in animals there are (normally) no post-maiotic divisions, whereas in plants there may be, and often are, a large number. In a fern, for example, the whole prothallial generation consists of post-maiotic cells, and it thus becomes clear that there exists no necessarily direct relation between the maiotic divisions and the differentiation of the sexual cells or gametes.

Referring to the terms in common use, viz. "heterotype," "homotype," and "synapsis," we employ these as descriptive of incidents that invariably are present in the maiotic phase. The word "heterotype" is applied to the first mitosis as it was originally used by Flemming, and the synapsis represents that series of events which are concerned in causing the temporary union in pairs of pre-maiotic chromosomes, previously to their transverse separation and distribution, in their entirety, between two daughter nuclei. We restrict the term "homotype" to signify the second division in the maiotic phase, instead of extending it, as some writers have done in the case of plants, to include all post-maiotic mitoses.

Thus the scheme of the cellular life cycle in any animal or plant may be represented as follows :

Pre-maiotic Phase.	Maiotic Phase.	Post-maiotic Phase.
Occurs in animals and plants, and begins with the development of the fertilised ovum.	Occurs in animals and plants.	Occurs in plants (gametophyte of the higher forms). Normally absent in animals.

We further suggest the desirability of using definite terms in order to express and describe the diverse aspects presented by different classes of mitoses in a given animal or plant; and since in any cellular life cycle all the pre-maiotic and post-maiotic, as well as one of the two maiotic, divisions are essentially characterised by the longitudinal splitting of the mature chromosomes, these might, for descriptive purposes, be termed Anaschistic mitoses. Similarly, inasmuch as the characteristic feature of the other of the two maiotic divisions (usually, if not always, the first) is transverse as regards the mature bivalent chromosomes this division might be designated as Diaschistic.

As regards the words "heterotype" and "homotype," they are not really necessary if our term of maiosis be accepted. They could more simply be designated as the first and second maiotic divisions respectively. But inasmuch as they are so well understood, and so widely adopted, we have continued to use them in the sense as already defined.

The series of phenomena that for convenience may be included under the terms of "regeneration," "variation," and "heredity" have gradually come to be more clearly apprehended as resolving themselves into cell-problems. And in reflecting on the results of modern cytological investigations in this connection, it is impossible to escape the idea that in some way or other the nuclear chromosomes of an organism must be intimately related with the structural characters by which it is distinguished. The intricate sequence of changes undergone by the chromosomes during the phases of a nuclear division, coupled with the surprising degree of similarity betrayed in these respects between the cells of plants on the one hand and of animals on the other, renders it impossible to avoid the conclusion that a fundamental significance lies behind the

structural features that reappear at each division of the nucleus.

Again, the regular recurrence of a numerical reduction of the chromosomes in the maiotic phase, which is intercalated once in every normal life cycle, emphasises the importance of these bodies in a still higher degree. But although it becomes obvious that in the details of maiosis we may reasonably expect to find an important clue as to the nature of that relation which must exist between the chromosomes and the essential features of ontogeny, opinions are still much divided on matters of cardinal importance connected with the process.

As is well known, two conflicting classes of interpretation have been advanced to account for the phenomena witnessed during the maiotic divisions. The divergence of opinion is largely due to the extreme difficulty of disentangling the true sequence of the events that are proceeding in the intricate series of changes that constitute the mitoses in question.

The view that may first be briefly summarised is one which has found much favour, and especially with zoologists. Weismann long ago insisted on the theoretical necessity of a reduction division in connection with his views as to the relation of ancestral characters with material primordia. The investigations of Häcker, Rückert, and others gave a welcome support to Weismann's views, and seemed to prove that they accorded with actual facts. They showed, in the animals investigated by them, that during the prophase of the heterotype mitosis the spireme thread, instead of giving rise to the full number of chromosomes characteristic of the preceding cell-nuclei, only formed one half the number of these bodies. Each chromosome was therefore regarded as bivalent, and as consisting of two monovalent chromosomes of preceding nuclear generations. The two individuals constituting a bivalent chromosome were considered as being attached end to end. Furthermore, the entire bivalent chromosome suffered longitudinal fission, and the question to be decided lies in the exact determination of the method by which the daughter

chromosomes of the heterotype and homotype mitoses respectively are provided for.

Häcker considered that during the heterotype division the longitudinal halves of each bivalent chromosome were separated, exactly as happens during an ordinary mitosis. At the second (homotype) division, however, each chromosome (which is still bivalent) splits transversely into its monovalent individuals, and in this way is provided the mechanism of reduction postulated by Weismann. Rückert and others have sounded a less certain note as to the particular mitosis during which reduction is effected. They admit that it may occur in the heterotype mitosis. Now, if either of these two slightly differing views as to the general significance of the heterotype and homotype mitoses prove to be generally true, it is clear, in the first place, that the opinion of those who hold that the chromosomes are to be regarded as permanent and persistent entities gains a strong, if somewhat indirect, support. For the significance of the numerical reduction is clearly related to the restoration of the full number of chromosomes at the next succeeding fertilisation. And on the view just outlined above, reduction involves no loss of individuality, for it is effected by the migration of half the entire number of somatic (or pre-maiotic) chromosomes to each of the two daughter nuclei respectively.

The second view, which has been largely entertained by botanists and by some zoologists, explained the processes differently. During the later stages of prophase of the heterotype mitosis, an appearance strongly suggestive of a second longitudinal fission of the chromosomes may often be observed. This was believed to provide for the division of these bodies in both the heterotype and homotype mitosis. In each of these, then, the mode of chromosome distribution would be similar, and it would resemble in all essential respects the process as it occurs in an ordinary somatic division. And furthermore every precaution would seem to have been taken, during the prophase of the heterotype mitosis, to secure the utmost degree of similarity between the

chromosomes of each of the four nuclei that result from the two maiotic divisions.

But such an interpretation involves important conclusions, not only as to the nature of reduction, but also as to the kind of importance to be attached to the chromosomes themselves. For if it be really valid, it becomes impossible to consistently retain a belief in the permanence of the chromosomes from one life-cycle to another. It is obvious that if their number is thus periodically reduced to one half, and if the resulting chromatic elements are distributed to the daughter nuclei solely after duplication by means of longitudinal fission, the individual chromosomes that arise during the maiotic phase could not possibly correspond to any that existed in the nuclei of the cells previous to the incidence of this phase of reduction. The only hypothesis consistent with such a view would demand the previous longitudinal fusion in pairs of the original chromosomes, a view that has not been seriously held by any who have maintained the existence of two longitudinal fissions during the heterotype prophase.

Hence it would follow that during the prophase of the heterotype mitosis the chromosomes for the next generation must, so to speak, be formed afresh. That is, they are entirely reconstituted—out of the original matter perhaps, but with a complete rearrangement of substance that would preclude any idea of continuity in their organisation. And this is equivalent to a denial of the permanence of the chromosomes from one generation to another.

Such a view does not, of course, necessarily involve a similar denial of the equivalence of the somatic chromosomes, in which there is no numerical reduction, but it relegates the whole question to a position of subordinate importance. It is obvious that such a result must profoundly affect any conceptions as to the nature of the relation that may be supposed to exist between the chromosomes and the mechanism of heredity. For if the inherited and other qualities of an organism are to be associated in any way with the chromosomes, and if these structures have no persistent organisation

of their own, the supposed relation can at best be dynamical, depending on the chromosome substance as a whole rather than on that of the individual units. No doubt the connection of the nuclei with the specific organisation of the cell—or of the cell aggregates—is, in the last resort, almost certainly of this nature; but the whole problem turns on the question as to whether the discrete particles (chromosomes) are endowed with different activites, or whether each of them merely acts as a portion of a homogeneous whole.

Many à priori considerations appear to be opposed to the latter view, and seem strongly to point to a difference between the different chromosomes, each of which, by itself or in combination with others, can produce a definite effect in directing or influencing the latent activities present in the nucleus or the cell. The complex series of events during a normal somatic mitosis whereby an exact longitudinal division of the chromosome material is effected has often been commented on, and it is difficult to comprehend why longitudinal fission should be so invariable a rule in normal differentiating body cells, unless there is an individuality possessed by the chromosomes themselves—an individuality that would manifest itself in retaining or modifying the specific traits distinctive of the organism. Again, the remarkable constancy of numbers, especially in the reproductive tissues, fails to find any satisfactory explanation.

It is true that some, like O. Hertwig, have regarded equality of mass as the essential advantage secured by longitudinal fission, but this standpoint, from the point of view of the facts of ontogeny, seems an unsatisfactory one. The celerity with which two cells of common parentage may proceed to differ, in spite of the equivalence of their nuclear mass at the instant of their genesis, coupled with the rapidity with which nuclei may grow or diminish in size, are difficult facts to reckon with when regarded from this, comparatively speaking, simple standpoint. The results of experiments on regeneration of embryos and missing portions of older

organisms emphasise the importance of constituents, rather than of the substance regarded as a whole.

Again, the interesting results obtained by Boveri and others during a study of the effects of polyspermy, and the analysis of the subsequent behaviour of the supernumerary chromosomes in relation to abnormalities, further emphasise the individual importance of each of these structures, and tend to show that normal organisation depends, inter alia, on a normal grouping of chromosomes, and not on the presence of a mere normal amount of chromosomic substance.

Furthermore, a considerable weight of evidence has accumulated within recent years that renders it difficult to dissociate the facts of heredity from an admission of the existence of discrete particles that are, individually or collectively, responsible for the appearance of those particular traits that characterise one organism and separate it from others. Investigations on the behaviour of hybrids militate strongly against the assumption that during fertilisation any real fusion of the parental substances responsible for the expression of particular features occurs.

To avoid possible misconception, however, we may as well state expressly that in thus formulating the problem as it presents itself to our own minds, we are far from supposing that the "hereditary substance" may not operate correlatively, so as to become responsible for the production of groups of characters. But admitting that the chromosomes really possess the sort of importance usually assigned (on good grounds, as we think) to them, we fail to understand how a mixture, amounting really to complete fusion, of such hereditary substances can produce the observed appearances. How, for example, could one account for the segregation of ancestral characters in inter-breeding hybrids, if the individuality of the original chromosomes becomes really obliterated during each generation? But, on the other hand, as Weismann long ago pointed out, it is impossible to continue indefinitely to accumulate the primordia (anlagen) of characters, as they are doubled at each act of fertilisation,

within a limited and approximately constant mass of substance. Hence if we admit that the chromosomes divide longitudinally (anaschistically) throughout the maiotic, as in the somatic, cell generations, we are confronted with the following difficulties:

1. The reduced chromosomes cannot continue to be compounded of the antecedent premaiotic chromosomes and at the same time preserve their organisation unchanged. They must each represent a new structure. Why, then, under these circumstances do they appear strictly as half the number characteristic of the preceding nuclei? For if the equal division of the mass be the essential feature, there would seem to be no specific reason for constancy in respect of number.

2. If chromosomes arise de novo from the substance of the previous ones that have now lost their identity, the only result must be a mingling of substance, but no retention of organisation. But such a mingling cannot be simply of the nature of a mixture. It is more akin to the production of a new chemical combination at each reduction, since the parental masses of nuclear substance can scarcely be supposed to be absolutely identical, especially in the case of hybrids. But it is just in hybrids that we meet perhaps the strongest evidence in favour of the continued existence of the primordia as attached to discrete particles retaining their individuality, for how could the remarkable numerical relationships of dominants and recessives be otherwise maintained?

The difficulties briefly sketched above seem to render the existence of a double longitudinal fission during the mitoses in question not only inherently improbable but impossible to reconcile with the facts so strongly pointing to the important influence exerted by the separate chromosomes in controlling and determinating the organisation of an individual plant or animal.

Moreover, such a mode of fission, with the consequences that accrue from it, would afford no satisfactory explanation

of the series of changes that so constantly recur in the heterotype and homotype mitoses of animals and plants. For it is not apparent why the mere halving of the numbers should lead to events so peculiar and characteristic as are those prevailing during these divisions. It is, therefore, doubtful whether Hertwig's suggestion that the intrinsic importance of the two mitoses lies in the consecutive and sudden reduction of the chromatin to one fourth of its original mass, can be accepted, seeing that, in some cases at any rate, a lapse of no inconsiderable time may intervene between the termination of the heterotype and the onset of the homotype mitosis.

In short, the assumption of a double longitudinal fission as constituting the essential mode of division not only fails to explain difficulties arising out of comparative observations, but it raises others of a serious kind which are opposed to both observation and theory.

But in spite of the difficulties inherent in it, the view we have just discussed has been widely adopted as that most in conformity with the best observations. It appeared to have rested on a solid foundation, for example, in the special case of Ascaris, the spermatogenesis of which was carefully worked out by Brauer. Flemming and, more recently, Meves have repeatedly insisted on the absence of any appearance that could be conclusively interpreted in the sense of a transverse separation of entire chromosomes in the Salamander. We, ourselves, formerly shared the same opinion. But when one proceeds to critically examine the evidence on which it is founded, it becomes clear that, with very few exceptions, there are lacunæ in the descriptions. These omissions are noted to refer to identical stages, both in animals and in plants. Everyone may have carefully observed the early stages of prophase, but one constantly discovers that the description and figures hurry on to the later stages, in which the definite chromosomes can be fully identified. The intermediate steps are missed out, and this is due to the great difficulty which they present in the way of satisfactory fixing and subsequent observation and elucidation.

Thus much of the existing divergence of opinion relates to the interpretation to be placed on these later stages, although these cannot really be understood save by the study of an unbroken series. Naturally the omission was not intentional. But the later stages seemed to fit so well on to the earlier, that the necessity for special caution as regards the intervening ones was not apparent.

Speaking broadly, the longitudinal fission of the spireme (or its representative) has been very generally recognised, but the phase which has next attracted the largest share of attention has been that in which the chromosomes are becoming definitely segregated previously to the assumption by them of their mature form and their final congregation on the spindle.

With the hiatus that intervenes between these two phases we are not now concerned, as it forms the main part of the observations recorded in the body of this memoir, but we may briefly glance at some of the interpretations that have been put on the structure of the heterotype chromosomes themselves.

In the case of salamander and lily, as examples of an animal and plant respectively, the definite heterotype chromosomes exhibit the forms of rings, loops open at one end with the sides more or less twisted round each other, and finally, especially in the lily, of rods, lying either parallel or twisted round each other. These figures were easily referable to, and were supposed to be derived from, the split spireme thread by its transverse segmentation, and the more or less intimate union of the ends of the parallel halves of the transversely isolated segments with each other. Within the last ten years an increasingly large number of examples have been discovered in which the two "longitudinal halves" of each heterotype chromosome were observed to show signs of a fission, and this has been commonly interpreted as the second longitudinal fission preparatory to the further division of the chromosomes in the next succeeding (homotype) mitosis.

In another series of examples, of which Arthropoda (Rückert, Häcker, and others) and ferns (Calkins) may be

cited as examples, the processes seemed easier to interpret in another sense. The chromosomes appear as tetrad-like bodies, which separate as pairs of dyads in the heterotype, whilst in the homotype mitosis each dyad further divides into monads, which are thus distributed between the daughter nuclei at this (second) division.

It has been often maintained that these appearances indicate a true sorting of somatic chromosomes, i. e. is a qualitative reduction in Weismann's sense. The tetrads are admitted to have arisen as the result of a longitudinal, associated with a transverse, fission of the substance of the chromosome, each of the latter thus being a bivalent (Häcker) structure, and representing a pair of adherent longitudinally split somatic chromosomes.

One of the most important memoirs on this subject of reduction is that by Korschelt[1] on Ophryotrocha. He maintained that in the heterotype prophase the full somatic number of chromosomes appeared, and that these subsequently fused in pairs to form the reduced number. During the metaphase they again became separated from each other and distributed to the daughter nuclei, and thus the first (heterotype) mitosis was clearly a qualitative one. Korschelt's observations did not fall very well into line with the process as described for other forms by other investigators, and Wilson, in his work on the cell, comments on the isolated nature of the results. But our own observations, extending over a wide range of forms, of which a brief abstract has already appeared (1903), as well as the more recent results obtained by Strasburger (1904), show that Korschelt's results, obtained in Ophryotrocha, are susceptible of a much wider application.

In 1895 a paper was published by H. H. Dixon,[2] in which he suggested the existence of a reduction division arising by the distribution of the equivalents of entire chromosomes, but

[1] Korschelt, "Ueber Kerntheilung, Eireifung, und Befruchtung bei Ophryotrocha puerilis," 'Zeitschr. für Wiss. Zool.,' lx.

[2] 'Proc. Roy. Ir. Acad.,' iii.

his account failed to carry conviction because it was evident that he had either misinterpreted the longitudinal fission (which does actually exist) as due, not to fission, but to the lateral approximation of distinct parts of the spireme thread, or else he overlooked the fission altogether, confusing it with that approximation which really does occur at the later stage. To judge from his figures, the former alternative appears to express the real explanation of his results. Schaffner[1] in his investigations on Lilium philadelphicum undoubtedly gave a correct explanation, in all important respects, of the sequence of events so far as the reduction divisions of this plant are concerned. His results, however, did not meet with the reception they merited because they were overshadowed by statements respecting centrosomes which were in contradiction with the positive results of all the most careful work of that time.

Atkinson,[2] in a paper on the reduction divisions in Arisæma and Trillium published in 1899, stated that the reduction was qualitative, i. e. essentially consisted in the transverse division of bivalent chromosomes. But he suggested that in the former plant the process was accomplished during the heterotype, whilst in Trillium it occurred during the homotype, mitosis. We have had the opportunity, through the kindness of Professor Atkinson, of examining some of his slides illustrating each of these plants, and we are quite in agreement with him as far as Arisæma is concerned. With respect to Trillium, however, the material at our disposal did not enable us to reach a definite conclusion; but we are strongly inclined to think that in this plant also the qualitative division is accomplished during the heterotype mitosis, and we are strengthened in this by a study of the excellent series of figures given by Ernst[3] in his memoir dealing with Trillium and Paris. The appearances are essentially similar to those met with in Lilium; and

[1] 'Bot. Gazette,' vol. xxiii.

[2] *Ibid.*, vol. xxviii.

[3] Ernst, 'Flora,' Bd. xci.

though Ernst himself decides in favour of a double longitudinal fission, we feel but little doubt that a renewed investigation will show that the chromosomes are really bivalent. An inspection of Fig. 5, Pl. 34, of his memoir strongly supports this suspicion.

Montgomery,[1] in a series of papers of which the most important appeared last year, describes a state of things for the amphibia investigated by him which is in complete accord with the conclusions arrived at by ourselves. We were unaware of his investigations when our preliminary note was published, and his paper only came into our hands afterwards. It is gratifying, however, to find that another investigator, working quite independently, had arrived at conclusions precisely similar to those which our own extended series of researches on critical examples, both of animals and plants, had led us to adopt as a general interpretation of the phenomena of reduction. More recently, Williams, in working out the cytology of the reproductive cells in Dictyota, and also Gregory, who has investigated the genesis of the spores of a number of ferns, have each arrived at results that are concordant with those put forward by us in the paper already referred to.

In a recent paper by Jules Berghs,[2] an attempt is made to sustain the older view for the cases of Allium fistulosum and Lilium lancifolium. We have ourselves examined the latter plant, and we are quite unable to concur with M. Berghs' conclusions. We readily agree with him that it is entirely a "question de sériation," but we cannot agree with him that it is possible, at any rate except in most exceptional cases, in one anther lobe to obtain anything approaching to complete sériation of the stages to be found in a single loculus. It is indeed just to his assumption of such a possibility that we attribute M. Berghs' error of inter-

[1] Montgomery, "The Heterotype Mitosis in Amphibia and its General Significance," 'Biol. Bull.,' iv, 1903.

[2] Berghs, J., "La Formation des Chromosomes Héterotypiques dans la Sporogénèse Végétale," 'La Cellule,' t. xxi.

pretation. A simple inspection of the figures that accompany and illustrate his paper suffices to show that the very stages that we regard as of critical importance are lacking. Moreover, his drawings do not carry conviction. They are either very schematic, or else they are based on preparations in which all the finer details of structure have been inadequately preserved. And finally, in the text, he gives no evidence of having paid special attention to the admittedly difficult stages which alone contain the solution of the problem.

As long ago as 1894 Belajeff, in a paper published in 'Flora,' on Iris and Larix, maintained that a true reduction occurred in these plants. But he was led, by the emphasis laid by him on the figures exhibited during the later stages of the process, to attribute the real reduction (qualitative) to the homotype mitosis, just as some of the Freiburg investigators had done. Strasburger and others have since shown this position to be untenable, and the conviction has slowly grown up that the second (homotype) mitosis—in plants, at any rate—is certainly associated with a longitudinal fission, and not with a transverse or qualitative distribution.

As these lines are being written we have received from Professor Strasburger[1] a memoir dealing with reduction divisions. The results are in substantial agreement with those contained in our previous communication, and which are here presented in an amplified form. The case of Galtonia, as described by Strasburger,[2] is especially in-

[1] Strasburger, E., "Ueber Reductions Theilung.," 'Sitz. ber. d. K. Preus. Akad. d. Wiss.,' 24 März, 1904.

[2] We note on p. 6 of the separate copy that the author seems perhaps to have not quite understood our position, as taken up in the preliminary note read before the Royal Society. The closed rings (geschlossene schleifen) were described by us being most common, but our diagrammatic fig. 4, in the note referred to, shows clearly one bivalent chromosome with both ends free, which proves we had not overlooked these cases. The regularity of the loops is much greater in animals than in plants, hence perhaps the emphasis that was put upon these figures in the note, which had very briefly to indicate the general results of the investigation as a whole rather than to discuss details.

teresting, since it puts the facts of reduction for this plant in a light as diagrammatic as Korschelts' investigations had already done for Ophryotrocha.

Perhaps one may venture to suggest that the Arthropoda, and other forms, in which the transverse division has been assigned to the homotype mitosis (Häcker and others) are worth re-examination from the new point of view. It must be remembered that the location of the transverse plane of separation in a symmetrical tetrad is not an easy matter; and the assertion that, in the heterotype mitosis, it lies in the longitudinal axis of the spindle, can only be maintained provided it can be shown that the developing chromosome retains its primary orientation unchanged from the time at which the transverse and longitudinal planes could be distinguished. Otherwise some unaltering mark is required to enable the observer to fix the planes in some other way. The difficulty of deciding as to the particular plane affected is at once rendered obvious on reflecting how the remarkable movements of the chromosomes themselves, just prior to their congregation on the spindle, may affect their ultimate orientation.

We have made no pretence, in this brief introduction, of dealing exhaustively with the immense mass of literature that has grown up around the problems connected with reduction. That formed no part of our task. We desired merely to indicate some of the principal trends of opinion in these matters, and to point out that it is plainly desirable to ascertain whether or no some reconciliation between the various conflicting views may not be possible. For when one reflects on the widespread occurrence of the phenomena in question, extending as it does to all the metaphyta and metazoa (if we exclude certain suggestive cases of parthenogenesis) it is clear that we are in the face of a fact of fundamental importance, whatever its true significance may ultimately turn out to be. And furthermore, our own comparative studies of karyokinesis in plants and animals, extending over many years, have impressed us with the remarkable similarities

that characterise the reduction divisions in the representatives of both kingdoms alike. We are convinced that it is highly improbable that these obvious similarities mask any fundamentally important differences.

The extreme orderliness to be observed in the whole process strongly suggests that in both kingdoms the true sequence and the actual nature of the processes involved will turn out to be identical. Otherwise the very orderliness of the process finds no meaning. And if it be true, as we believe it to be, that we can gauge the importance of phenomena in the organic world by the regularity of their appearance and procedure, then it would be difficult to discover any instance that more amply fulfils the required condition than do these complex series of changes involved in an ordinary nuclear division, as well as the no less remarkable and constant deviations from it that characterise the heterotype mitosis.

The results of our investigations, set forth in the following pages, have been such as to convince us that so far as metazoa and metaphyte are concerned, a real similarity between the process of reduction, as it occurs in animals and plants, does obtain.

The reduction is achieved by the association or by the non-separation of somatic pairs of chromosomes during the heterotype prophase.

The heterotype mitosis essentially consists in the separation and distribution between the daughter nuclei of entire somatic chromosomes, the separate identity of which is masked by their temporary union previously to the onset of the diaster, and thus the exact numerical reduction is accounted for.

The homotype mitosis is associated with the completion of the longitudinal division of the chromosomes already incepted during the prophase of the heterotype division.

If (as in many plants) there be post-heterotype cell generations, the reduced number of chromosomes is retained until the occurrence of nuclear union at fertilisation.

Detailed Description of Typical Examples of Animals and Plants Investigated.

I. Lilium Candidum.

The development of the spores in different species of lilies has so often served as the subject of investigation that it might seem but slightly probable that any fact of material importance still remained generally unknown. It has already, however, been remarked that divergent views as to the course of events during the heterotype and homotype mitoses in these plants have been advanced, and the matter cannot, therefore, be regarded as yet to be conclusively settled. Whilst the majority of observers hold that a longitudinal division of the chromosomes obtains in both the homotype and the heterotype mitoses, Schaffner[1] has adduced evidence in support of a "reducing" (*i.e.* transverse) division occurring in the heterotype, whilst Dixon[2] has considered that this was achieved during the homotype division.

The principal evidence relied on by those who advocated the existence of a longitudinal fission in each mitosis has been the supposed proof of the existence of a double fission during the late prophase stages in the heterotype. The more recent work of Grégoire and others appear to show conclusively that at any rate the homotype mitosis does not, in lilies, effect a transverse separation of chromosomes, but merely consummates a longitudinal fission already incepted during the early stages of the preceding mitosis.

We have also studied the homotype division in lilies afresh; and whilst in certain points our views diverge from those held by most other investigators, we still consider that the most important features of this mitosis consist essentially in the separation and subsequent distribution to opposite poles of equivalent halves of the chromosomes, and that these equivalent halves had already been marked out and defined

[1] 'Bot. Gazette,' *loc. cit.*

[2] 'Proc. Roy. Ir. Acad.,' iii.

during the earlier stages of the preceding (heterotype) mitosis.

When one turns to the first maiotic (heterotype) division itself, the case is widely different, and it is a singular as well as a somewhat unfortunate circumstance that a genus offering such special difficulties in the way of correct interpretation of the sequence of changes should have been so constantly and often exclusively studied by those who have generalised on the events that obtain during the course of the mitosis in question. For even in a single anther the temptation to regard the series as therein presented as representing a transitional series of phases has misled some writers. It very seldom happens that any such a complete series that embraces the critical, but transient, phases can really be so traced; and, moreover, some of these important phases are often not easy to fix satisfactorily, perhaps just on account of their changing character.

As the result of an examination of a very long series of preparations, illustrating the processes in a number of species, we have been irresistibly driven to the conclusion that the evidence for the existence of a transverse (reducing) division during the heterotype mitosis is irrefragible, and we think we are in a position to explain the sources of the more important differences of opinion expressed by others who have worked on these plants.

At the conclusion of the last archesporial division of the sporogenous tissue the nucleus goes into a state of almost complete rest. The chromatin exists as scattered granules, though here and there a thread-like arrangement can be seen (Pl. 34, fig. 1). The great bulk of the staining matter in the nucleus is, however, concentrated in the nucleolus, of which there may be one or more in each nucleus. As yet the archesporial cells are closely coherent, but as they increase in size intercellular spaces begin to appear at the angles where several cells meet. About the same time the linin becomes more chromatic, and in the majority of cases the general impression is conveyed that this increase in chromatin

is connected with changes in the nucleoli. The linin framework becomes more and more clear, but at first it is impossible to make out in it anything suggesting a continuous thread. Rather it appears as a large number of fibrils irregularly arranged in groups (fig. 1). Attempts were made, though without decisive results, to ascertain whether the number of these groups bore any definite relation to the number of chromosomes. In some cases there appeared to be such a correspondence. The outline of the individual linin filaments is irregular, and staining droplets of a chromatin-like substance, possibly of nucleolar origin, are often found adhering to them. Perhaps this substance may be regarded as equivalent to the "basichromatin" of some authors.[1] The general appearance exhibited by the nucleus at this stage is that of a sphere containing, besides the more or less numerous nucleoli, a grumous precipitate which tends to become aggregated in delicate fibrils.

From these fibrils the linin spireme arises. It appears, in uninjured nuclei, to form a continuous thread, although it is difficult, owing to the numerous convolutions of the skein, to be quite certain of this. It is of course impossible, save from the continuity of stainable substance, to form any valid judgment as to the nature of the spireme as to whether it is continuous or otherwise, and it may be that the appearance of isolated fibrils in the previous stage is really due to lack of equidistance in the arrangement of the chromatin. In other words, it may be that a continuous thread of linin does really exist in this earlier stage, although we have not been able to identify it as such, and for the present do not feel disposed to assume more than the appearance observed seems to warrant. Perhaps the matter is not one of great importance, for it is at any rate certain that at the close of the previous di aster no such continuous filament was present.

But the definite spireme thread can be distinguished very clearly at an early period in karyokinetic activity, certainly long before the spore mother-cells dissolve their union with

[1] Heidenhain, 'Ueber Kern and Protoplasma,' 1893.

each other. It forms a colourless thread, at first infiltrated with chromatin throughout, but the latter soon collects into serial beads so as to give rise to the well-known alternation of stainable (chromatin) and non-staining (linin) discs. The numerous small nucleoli previously seen have disappeared and become replaced by one or more relatively large ones. At first irregularly coiled in the nucleus, the differentiating spireme next aggregates towards one side, and there forms what we may designate as "the first contraction figure" (Fig. 2). The thread becomes densely coiled in the vicinity of the nucleolus, exhibiting a highly characteristic arrangement. This figure has often been dismissed as the result of imperfect fixation, but there exists strong evidence to show that it represents a normal occurrence in the life history of these cells. Miss Sargant states she has observed it in the living spore mother-cells of lilies, and we have not unfrequently seen it in the corresponding cells of Tradescantia, Osmunda, and several Liverworts, as well as in some animal spermatocytes. It is a style that persists for some time, but as it passes away the filament again becomes more loosely coiled and diffused, especially about the periphery of the nuclear cavity. It is perhaps a fact of some significance that the nucleus at this stage is relatively large, the average diameter in the case of pollen-mother-cells of Lilium candidum being 32 μ, as compared with diameter 29 μ reached by the nuclei at the contraction-figure stage just described.

A certain degree of polarity is observed to characterise the spireme thread as a whole at this stage, for the convolutions are absent from, or at least scarce in, one region of the nucleus, and this seems to be related to the emergence from the stage of contraction. The region of comparative freedom from convolution is about diametrically opposite to the spot at which the aggregation previously had occurred.

The longitudinal fission of the thread is now to be seen (figs. 3, 4). At first the beads or discs of chromatin lengthen out somewhat in the plane of cross-section of the thread;

then they are seen to be furrowed and to assume a dumb-bell-shaped appearance. Finally the halves of each bead separate from one another and come to lie in two parallel rows at the edges of the flattened spireme ribbon.

The ribbon itself next splits longitudinally. The fission is irregular, especially at first, and it merely forms open loops, closed at either end where the ribbon has not yet split. But later on it becomes much more complete and the halves proceed to divaricate (Fig. 5) more or less considerably from each other. This fission has been more or less clearly recognised as such by most writers who have investigated lilies, with the exception of Dixon, who regarded the appearance as due to an approximation of originally separate filaments. In the lilies the result of fission is much more marked than in the majority of other plants studied by us. It is doubtless to this circumstance that the prevalent misconception as to the true nature of the succeeding changes is due, and it serves to emphasise the necessity of comparative study as opposed to an undue reliance on the results of investigations made on single types, however promising these may individually seem to be. Thus a comparison of the processess as they are manifested in the lily with those corresponding to them in the Osmunda, Tradescantia, or Aneura, at once throws light on the actual sequence of events, though the investigation in no case is an easy one. But the evidence is quite decisive, and indicates re-approximation of the separated halves of the ribbon. Thus the split gradually closes up again (Figs. 7–11) and may be so nearly obliterated as to become very difficult to recognise. At the same time the thread is shortening and thickening, whilst the polarisation already alluded to may be more easily seen. The thread, in many of its convolutions, is attached rather securely to the nuclear wall, whilst the rest becomes aggregated into a somewhat dense tangle towards the centre, where the nucleolus is now commonly situated. The latter body (there may be one or more of them present in each nucleus) is vacuolated and has clearly lost much of its substance. This has been utilised

in the development of the chromatin element in the spireme, as is shown both by staining reactions, and by its intimate relation with the spireme during the progress of differentiation and growth of the latter. About this time the nucleus attains to its largest size, 35 μ being an average measurement of the diameter in Lilium candidum. As the contraction proceeds, which it does with great rapidity, the original longitudinal fission ceases to be noticeable and is only visible in exceptionally favourable cases. But a rearrangement of the thread, first correctly explained by Schaffner in the case of L. Philadelphicum, now sets in. Parts of the thread forming the spireme become pulled into parallel positions. This is specially well seen in those places where at the bend of a convolution an attachment to the nuclear periphery has taken place. Often the nuclear wall is drawn inwards at these spots. Thus a close and parallel approximation of lengths of the entire spireme thread is effected, and this parallel arrangement has been commonly interpreted as representing the parallel split halves of the spireme thread. Such an interpretation is, however, shown to be unsound by a careful study of the stages just described. Sometimes in one or both sides of the narrow V-shaped figures thus produced the original fission can still be traced, and this is especially the case when free ends of the thread can be observed. For at this time, and possibly earlier, the definitive chromosomes begin to be recognisable, though often each one is still connected by strands of linin with those lying next to it. This relic of the original fission has been recognised by others, but it has been commonly interpreted as due to the occurrence of a second longitudinal fission. No such second fission, however, really takes place at all.

As a consequence of the bending over of the spireme thread, or rather parts of it which give rise to the chromosomes, the segments when isolated very often exhibit the form of a loop, open at one end, with sides either parallel to each other or, more commonly, twisted over one another (Figs. 9, 11). But it by no means follows that all the bivalent

chromosomes are formed in this way, and as a matter of fact they are not. Sometimes two more or less straight rodlets become approximated with or without interlacing, whilst at others the ends of the rodlets may unite together so as to give rise to figures of rings, ellipses, etc. These various figures (c f. Figs. 11–13) may originate in various ways, and it is not necessary to discuss them more fully.[1] The important point to bear in mind is this, that the two rods, sides of loops, or whatever other form the structure as a whole may assume, represent, not the longitudinal halves of a split thread, but the approximation of serially distinct regions of the spireme as a whole. Thus each heterotype chromosome is a bivalent structure, and their "reduced" number (i. e., half that of the somatic chromosomes) is due to the approximation and more or less intimate, though temporary, union of the equivalents of pairs of somatic chromosomes.

It will be convenient to speak of the compound (paired) structures which are thus formed as chromosomes, although it must be remembered that each is in reality a double or bivalent body. As they become shorter and thicker, they become more homogeneous, and all trace of the primary fission (second fission of other authors) becomes completely obliterated. The nucleus shrinks in size, now measuring about 30 μ in diameter. The nucleolus, although it has lost much of its substance, is still recognisable as a large, often irregularly-shaped body, or it may have fragmented into a number of smaller pieces. A very characteristic phase then comes on. The chromosomes act as though affected by a mutual repulsion, and instead of being more or less massed together towards the centre of the nucleus, they move apart and lie at the periphery of the nucleus, the nuclear wall becomes thinner, and nucleolar matter escapes from the nucleus into the cytoplasm. Often, indeed, it seems as if it were forcibly ejected.

The characteristic cytoplasmic radiations now appear,

[1] Cf. Farmer and Moore, "On the Essential Similarities existing between the Heterotype Nuclear Divisions in Animals and Plants," 'Anat. Anz.,' 1895.

starting, as has been observed by ourselves and others, from many centres. The radiations, however, soon become more definitely polarised, and the nuclear wall, often at this time showing irregularities in contour, gradually disappears, and the chromosomes become grouped in the equatorial plane. At first they are irregular in their arrangement, but soon exhibit the well-known definite plate-like arrangement. The achromatic spindle-fibres are very clearly differentiated during the movements referred to, and they give the impression of actively driving the chromosomes to their final equatorial positions. We do not adopt this view of their nature, as we believe them to represent protoplasm modified by the forces at work in the cell rather than actively growing entities that are spontaneously concerned in producing the movements in question. Thus we consider that the movement is produced by the same causes that operate so as to differentiate the spindle. The latter appears then as a passive manifestation of the real operating agency, rather than an active director of the movements in question.

Outside the area occupied by the chromosomes isolated spindle-fibres, or groups of such, are seen to diverge from the main polar directions and to end upon deeply staining droplets of nucleolar origin. This fact, long ago pointed out by one of us[1] (1893), is of special interest as bearing on Strasburger's view of the connection of the nucleolus with kinoplasm.

The individual (bivalent) chromosomes assume many different forms on the spindle, as has already been pointed out by us in a previous paper;[2] but during the metaphase one general mode of procedure is seen to govern their division. Each bivalent chromosome divides so as to separate monovalent elements, which are then distributed to the respective poles. The mode of separation varies in the case of different chromosomes, the difference depending on the manner in which the latter are arranged with

[1] J. B. Farmer, 'Annals of Botany,' vol. vii, 1893; cf. also 'Flora,' 1895.

[2] "On the Essential Similarities existing between the Heterotype Nuclear Divisions in Animals and Plants," 'Anat. Anzeiger.,' 1895.

reference to the spindle-fibres, i. e. to the forces that effect their final separation. In the majority of cases a chromosome is as a straight or twisted structure, projecting radially from the equatorial plane. Then each monovalent half is attached at or near one end to a sheaf of achromatic spindle-fibres, and the two halves (i. e. the monovalent constituents) of each chromosome slide over each other and travel towards the appropriate pole. As soon as this migration commences the longitudinal fission once more becomes apparent, and the rod splits open along the greater part or even the whole of its length, so as to give rise to the V-shaped daughter chromosomes. Each limb of the V represents the original half of the spireme thread that was formed during the prophase. Grégoire [1] was the first to recognise that this V-shaped form is due to the re-opening of a previously effected longitudinal fission. But he considered that two longitudinal fissions occurred during the prophase, and that the appearance in question was due to the re-opening of the second of these. Although we cannot accept the interpretation in that form, since we have shown that the supposed second split really represents the first (and only) one in a disguised form, it is obvious that Grégoire was correct in his main contention, viz., that the production of the V depended on the re-opening of a previously effected fission. And the interpretation receives a striking confirmation from certain types of chromosomes that are occasionally to be observed in the diaster of lilies. The chromosomes in question assume the forms of V's, but each is seen to be completely split throughout its entire length. Such a figure is produced when a heterotype chromosome becomes attached by the middle instead of by the end, to the spindle-fibres (*cf.* Figs. 15, 16, 17). The whole daughter chromosome is then bent over into a V-shaped structure instead of forming a rod-like body. Hence the longitudinal fission, on its re-appearance, gives rise to the figures of split V-shaped bodies.

[1] V. Grégoire, "Les Cinèses Polliniques chez les Liliacées," 'La Cellule,' t. xvi.

Although such figures are rare in the lily, they are quite common in Tradescantia, and also in the salamander, as was long ago figured and described by Flemming. The same interpretation, as will be apparent from what follows below, is also applicable to such cases.

When the daughter chromosomes arrive at their respective poles the nuclei are reconstituted, and a complete bipartition of the pollen-mother-cell takes place. It is not necessary to give details of these processes here, as they are not relevant to the main object of the paper.

The nuclei do not pass into a state of complete rest, although it is not practicable to trace with certainty the individual identity of the chromosomes throughout the whole period intervening between the appearance of the nuclear wall and the next mitosis. But enough can be seen to leave no doubt as to the course of events that characterise the second (homotype) mitosis of the spore-mother-cell.

As the chromosomes for this second (homotype) mitosis disentangle themselves from the chromatic plexus of the nucleus, they are found to present some diversity in form, and this is continued up to the stage of the diaster.

Often they look like sinuous V-like structures with the ends thicker than the middle. The limbs of the V are long, and finally break asunder at the bend. The two halves then separate, but usually show a crook or curvature where they separate. Finally the respective limbs diverge one towards each pole. In other examples the chromosomes appear as longitudinally split V-like bodies. These are to be related with the similar structures seen as occasional varieties during the diaster of the preceding heterotype mitosis. Both these forms have long been familiar to us, and have been observed by others, but it is clear that they are only special cases of the general phenomena. But the former and much more commonly occurring forms have been regarded by some, e. g. Belajeff,[1] as indicating the existence of a transverse fission during the homotype mitosis, and thus as proving

[1] 'Flora,' 1894 (Erganzungsbd).

that a true reduction division was associated with this particular karyokinesis. After what has been said it will, however, be clear that there is no real difference between the two cases, but that the second (homotype) mitosis results in the separation of the longitudinal halves of the original spireme thread that by their partial divergence have already given rise to the figures of V and Λ duringthe previous diaster.

Since the preceding account of the heterotype and homotype mitoses in Lilium was written, a paper has appeared from the pen of Professor Grégoire[1] in which he contests the correctness of the interpretation advanced in our preliminary communications last year. Professor Grégoire has considerably altered the views previously expressed by himself as to the actual sequence of events during the mitoses in question, and he cites in support of his present position some as yet unpublished work of his pupil M. Bergh. We think it desirable to examine the evidence for the views he now seems to hold in so far as they are set forth in his last paper.

He divides the prophase stage of the heterotype mitosis into two phases, the first extending from the commencement of the process and terminating with the formation of the thick spireme (spirème épais), the second beginning with this phase and culminating in the formation of the definitive chromosomes. After the first differentiation of the chromatic filaments by the breaking down of the alveolar arrangement which previously was associated with the distribution of the chromatin in a reticular-like way throughout the nucleus, the synaptic contraction sets in. Most of the filaments are indistinguishable, but those that can be identified are thin. In several places filaments may be seen to run parallel, sometimes twisted (entrelacées) and finally the two thin threads fuse to form a thick one. Following on this is seen a thick continuous spireme thread which disengages itself from the synaptic contraction and spreads through the nucleus. Soon a "longitudinal fission" appears in the thread, but he con-

[1] V. Grégoire, "La Réduction numérique des Chromosomes et les Cinèses de Maturation," 'La Cellule,' t. xxi.

siders that the split really represents the separation of the threads that have just before fused. The longitudinal fission therefore, strictly speaking, would not exist. The separated halves of the "thick spireme" contract and give rise to the two halves of each bivalent chromosome, when, by the transverse segmentation of the spireme thread, they can be identified as distinct individuals.

We have tried to state M. Grégoire's position as fairly as we can, and if we have correctly apprehended his meaning we find ourselves wholly unable to agree with him.

It appears to us that two series of events have been confused. There is not only one, but there are two contraction figures. In the first one, which Professor Grégoire seems to regard as the synaptic figure, we have been able to trace the spireme continuously; and there cannot exist the slightest doubt but that, as it emerges from this figure, the longitudinal fission occurs as we have described. It seems to us that Grégoire (and Berghs) has either omitted to observe the fission and has only seen the re-fusion of the split thread, or else he interprets the earlier stage in which the fission is as yet incomplete in a sense opposite to that in which we, together with most other observers, regard it. But it is rather difficult to follow the account given by Grégoire, inasmuch as he makes no mention of the second contraction (which we regard as the essential synaptic one) wherein the lateral approximations of the spireme occur. For we can hardly suppose that this contraction can have been confused with the earlier one, and yet apart from some such assumption it is difficult to reconcile the differences between our results. Moreover, Grégoire's account of course excludes the existence of a longitudinal fission in the approximated lengths of the now differentiating chromosomes, since he identifies these lengths with the products of that "longitudinal fission" (approximation according to him) which occurred at an earlier period. And yet traces of this fission can be seen at all the stages under consideration.

M. Grégoire appeals to the figures in M. Berghs' memoir

in support of his views, but we have already expressed our reasons for regarding them as inadequate to afford a complete picture of the whole series of changes.

The main points of difference between us are these:

1. M. Grégoire considers that during (?) the "synaptic" (1st) contraction a lateral approximation of thin spireme thread occurs, and that this then fuses. Our view is the reverse of this.

2. The closed, jointed threads next split asunder, and the doubled segments of the spireme thus formed give rise to the definitive chromosomes, with their variously twisted limbs. We regard the original longitudinal fission as temporarily closing; this is followed by an approximation of the thread into parallel lines, whether this is formed by looping or otherwise. At this stage the *second* contraction figure is intercalated. We find traces of the longitudinal fission to occur in the collateral threads from the first, whilst Grégoire does not admit its existence till after the chromosomes are arranged in the spindle.

M. Grégoire is in agreement with us in regarding each chromosome as a bivalent structure, and as equivalent to two somatic chromosomes lying in close juxtaposition or even partially united; and further, that during the heterotype mitosis a distribution of entire somatic chromosomes takes place.

II. Osmunda regalis.

The archesporial cells in the sporangium are characteristic in their appearance. The cells are large and somewhat oblong, and the very prominent nucleus is commonly placed excentrically, being nearer one end of the cell than the other.

The nucleus possesses a well-defined wall, and contains a nucleolus. The chromatin can certainly, at least in the early stages, be said to exist in such an arrangement as to suggest a spireme. Sometimes the granules of chromatin appear to be scattered irregularly, so as to give the impression that one is confronted by a foam structure, the granules lying

in the angles where the walls meet; whilst at other times these granules can be traced as lines or rows for short distances within the nuclear cavity. The regular spireme arrangement is thus the result of a progressive differentiation, a result encountered in other cases, e. g. in Tradescantia, and less prominently perhaps in Lilium.

As the spore-mother-cells approach maturity the chromatin assumes a more regular arrangement, and the linin framework begins to stand out more clearly from the paralinin that surrounds and encloses it. The thread now forms a thin, much-convoluted filament which seems to be continuous, though free from the cross anastomoses present at an earlier stage. At least no free ends could be with any certainty discovered. The chromatin is now very distinctly arranged in a single serial row of granules in the linin. At this stage the first contraction figure is to be met with. The coils of the spireme are densely aggregated at one side of the nucleus, but some parts of the whole thread remain free from the general tangle. Gradually the dense mass again becomes looser, and the thread rapidly shortens and thickens, whilst at the same time the chromatin granules are seen to be larger, though whether their increase in size is due to fusion, or, as seems more probable, to growth, could not be decided. Here and there signs of the longitudinal fission become apparent, inasmuch as single granules are replaced by double ones that lie in pairs along limited lengths of the thread (Fig. 22). The latter is still much convoluted, and its windings can easily be traced just beneath the nuclear wall. The longitudinal fission just mentioned does not become emphasised as in the case of Lilium, and the thread does not separate so distinctly into two longitudinal halves as in that genus.

The second (synaptic) contraction figure now sets in. The thickening thread gradually becomes massed together in the vicinity of the nucleolus, but distal loops are still easily seen which extend, and may be attached to, the nuclear wall. In these looped portions the signs of longitudinal fission are very

clear (Fig. 23). The sides of the loops become drawn into parallel positions as the tangle increases, and at the same time the nucleolus suffers a considerable loss of substance, as is evidenced by its vacuolation at this stage.

The sides of the loops just described continue to approximate more closely together, and thus simulate an appearance of a longitudinal fission. It is quite clear, however, that this appearance is illusory, for the real fission can often be traced in their parallel sides (Fig. 24) even at a much later stage.

Gradually the tangle around the nucleus vanishes, and the chromatic filament is then observed to have segmented transversely so as to form the definitive chromosomes. The actual process of transverse separation is somewhat slow, for all stages can be followed in suitable preparations. The stainable substance (chromatin) seems gradually to become attenuated so as to give the impression of a viscous body being pulled asunder.

It is very clear that much nuclein or chromatin has been withdrawn from areas of the original filament, for considerable tracts of the linin thread can be seen to evince no affinity for basic aniline dyes, and it often happens that these unstained lengths can be traced as being in direct continuity with others in which chromatin is abundantly embedded.

Although the parallel arrangement of the chromosome constituents may be provided for in the way just described, namely, by the approximation of the sides of an originally looped structure, this by no means exhausts the variations by which the same appearance can be produced. Sometimes long, rod-like forms with a slight bend in the middle are met with, and at others it seems as if the parallel arrangement of the sides is certainly affected by the approximation of two portions of the thread (Fig. 25) that have broken apart from each other. In fact, many different forms are to be seen, often in the same nucleus. The U-shaped loop is perhaps the most common, and a simple variation of this is produced when the sides or limbs of the loop are twisted round each other; at other times rings or ellipses are en-

countered. These become much more frequent at later stages, and they clearly owe their origin to the fusion of ends previously free from each other. Again, the two sides may be twisted over each other whilst both ends remain disconnected.

Meanwhile the spore-mother-cells have become completely detached from each other by the solution of the middle lamella, and the excentric position of the nucleus is strongly marked. A curious appearance is seen in each cell, at this and earlier stages, in the vicinity of the nucleus. In the cytoplasm at the narrower end of the spore-mother-cell a remarkable vacuolar arrangement of the fibrous cytoplasm is regularly seen as a highly characteristic feature that persists through the greater part of the whole stage of prophase (fig. 23). It seems to have nothing to do with the spindle formation that occurs later, and without hazarding any theory as to its significance, it may perhaps be suggested that it possibly indicates a withdrawal into the nucleus of substances previously contained in the extra-nuclear cytoplasm. As the formation of the definitive chromosomes proceeds, rapid changes begin to affect the tapetal tissue. The cells composing this nutritive layer have become enlarged, and the nuclei have multiplied, first, mitotically, and later on by an abbreviated process more akin to amitosis. The cell walls ultimately break down, and the cytoplasmic contents, together with the nuclei, escape into the interspaces between the spore-mother-cells. The nuclei long retain that curious condition of prophase so characteristic of the nuclei of many actively secreting gland-cells. Gradually, however, they undergo disintegration in the slimy mass that now fills the interstices between the separated spore-mother-cells.

Meanwhile the chromatic thread has segmented with the definitive chromosomes, or if previously in reality discontinuous, it at least now can be certainly so recognised. Many of these young chromosomes consist at first of U-shaped loops, with sinuously curved limbs. Sometimes the limbs are twisted round each other, and the impression is conveyed to

the observer that this twisting increases and becomes more prevalent in the following stages. The chromosomes now shorten rapidly and attain their final shapes, but the original longitudinal fission can often be traced quite distinctly in the thick limbs. The remains of the nucleolus may also be still recognised amongst the chromosomes, and indeed it does not really disappear until after the chromosomes become arranged in the equatorial plane of the spindle.

Immediately before the latter event takes place the chromosomes are, as is so common at this phase, distributed over the periphery of the nucleus just within the wall. They are thus in a specially favourable position to enable the relation of the various forms to one another to be traced. Speaking generally, the shape assumed depends very much on the character of the primitive or young chromosome as it emerges from the synaptic contraction (figs. 26, 27). The commonest forms are those of X, O, and 8. The last are easily derived from the U-shaped structure, whilst the figures O are due to the approximation and fusion of extremities previously free from one another. The very characteristic X figures may arise in several ways—either the spireme thread breaks up transversely into rods, and two of these approximate and cross, so as to form the shape in question, or they may have arisen from the 8-like chromosomes, by the complete breaking asunder and divergence of the limbs. Finally, it sometimes happens that the X-like form is produced by the approximation of two bent rods, thus:)(. A less commonly met with chromosome possesses the form of a long rod. This means either that a U-shaped loop has straightened out or that a piece of the linin, straight ab origine, is bivalent. Finally, it might arise, though we have no positive evidence as to this, by the end-to-end attachment of previously isolated segments of the spireme thread.

But these types very rarely maintain their individual characters up to the appearance of the spindle, and the great majority become transformed into X-like forms (fig. 28). It may happen that the monovalent constituents of many of the

bivalent chromosomes become almost or quite detached from each other about this stage. But they seem always to unite again before the completion of the spindle formation. The fact, however, is of interest, seeing that Korschelt has described, in the case of Ophryotrocha, an example in which the somatic number of chromosomes appears at the heterotype prophase; these then unite in pairs before they become finally arranged on the spindle.[1] The appearances here described for Osmunda are very plainly visible in many pleridophytes. Figures 29 and 30 illustrate corresponding phases in Psilotum triquetrum, a lycopodineous plant. When the chromosomes of Osmunda congregate on the equatorial plane of the spindle their differences of form become less marked; as they begin to separate on the commencement of the diaster, it is clearly seen that the division is a transverse one. Most of the chromosomes are more or less oval or diamond-shaped, but some retain the form of long rods that divide transversely across the middle.

The longitudinal fission so often recognisable in other plants at this stage is often difficult or impossible to distinguish, though it may be seen with certainty in some cases. The diaster is, as a whole, rather irregular. The daughter chromosomes cling together by one end equatorially, in a manner recalling that so often met with at the corresponding stage in Tradescantia. The way in which these rod-like chromosomes ultimately break asunder suggests a pull rather than a repulsion as the cause of their final separation, although the fact that the chromatin leaves the central zone when the final breaking occurs might perhaps be utilised as an argument to support the hypothesis of mutual repulsion.

At the close of the diaster the chromosomes can still be recognised as bands within the nuclear-wall which is formed before the onset of the next (homotype) mitosis.

The chromosomes as they become isolated and distinct at the

[1] Strasburger in his recent paper ("Über Reductionsteilung," 'Sitzber. d. R. Pr. Akad. d. Wiss.,' March 24th, 1904) has described a similar condition or Galtonia candicans.

commencement of the homotype division form, for the most part, rod-like bodies directed radially in the equatorial plane; often they are very clearly seen to be double at this stage, and when looked at from the side present the appearance of dyads. Some of the chromosomes are scattered through the equatorial plane, and are thus not confined to a peripheral position. As the daughter elements separate from each other they assume remarkable forms; the general impression obtained is that of viscous bodies forcibly pulled asunder. Thus they become very much attenuated and elongated as they finally separate and travel to the respective poles of the spindle. On reaching the poles they very rapidly shorten and thicken as the daughter nuclei pass into the state of telophase and ultimately of rest.

III. Aneura pinguis.

This species of Liverwort exhibits certain remarkable peculiarities connected with the formation and division of the spore-mother-cell that are absent from the corresponding mitoses of most plants. On the other hand, they are shared by most, if not by all, of the members of the Jungermannia series of Hepaticæ,[1] although in different degrees. At the close of the archesporial cell-divisions, as the individual cells become free from each other by the dissolution of the middle lamellæ, those cells that are destined to give rise to spores soon become differentiated from those that will ultimately form the elaters. At first the contour of each is irregularly spherical, but as they enlarge in size, it is seen that each spore-mother-cell becomes symmetrically bulged out at four spots, so as to form a quadrilobed cell. The lobes are arranged tetrahedrally, each diverging from the common centre, and thus the axis of no two or more of them can lie in the same plane. Hence it follows that it is necessary to exercise care in interpreting and combining the results of observations made on sections of such a structure. Aneura is, however, specially

[1] *Cf.* Farmer, "Studies in Hepaticæ," 'Annals of Botany,' vols. viii and ix.

favourable for study, inasmuch as, like Fossombronia, the lobes are not much extended in the radial direction, as is, for example, the case in Pellia.

The nucleus occupies the centre of the cell, and it is thus surrounded by, and enclosed in, cytoplasm which is chiefly aggregated into four masses corresponding with the four lobes already referred to.

The nucleus contains one or more nucleoli, and at this stage the spirem thread can be traced as a probably continuous filament within the nuclear wall.

The early contraction figure already described for the preceding plants occurs here also, but judging from the relative infrequency with which it was observed, it appears to represent a very transient phase.

As the nucleus begins to show signs of approaching mitosis, the first obvious change is seen in the cytoplasm. In each of the four lobes a centrosphere is differentiated (figs. 31–35), and sometimes a central body (centrosome) could be distinguished in each. The centrospheres when formed appear to exert (or to represent the foci of) tractive forces acting on the nucleus, which now changes its form and becomes distinctly drawn out, so that an angle projects towards each lobe. Before the formation of the centrospheres the nucleus was either spherical or even slightly flattened opposite each lobe. These facts can be made visible both in spore-mother-cells stained in bulk and mounted in glycerine, although of course the details can only be followed in sections. When sections are examined only three lobes at most can be seen at once, and unless the sections are fairly thick one can only trace fragments of the whole apparatus, since the axes of the centrospheres and spindles lie in four different planes. Aneura multifida, owing to the smaller size of its spore-mother-cells, affords a more favourable object in which to study the process in the unsectioned cell; and indeed that species, together with Fossombronia pusilla, is habitually used by us to demonstrate the quadripolar spindle and centrospheres to classes of students.

The spireme thread is much twisted and convoluted within the nucleus, and it shows longitudinal fission through considerable portions of its length (fig. 32). The fission is, however, very transitory, and it becomes even more obscured later on, through the fusion of the split halves.

The spirem now shortens and thickens, but the convolutions are still numerous—more so than the number of chromosomes ultimately to be produced. As the contraction proceeds, it is easily seen that in many places the loops of the spirem are adherent to the nuclear wall, and the latter may even be slightly pulled inwards at these spots. The chromatic thread rapidly becomes more rich in nuclein, the nucleolus contributing to this process and itself losing a large portion of its stainable constituent. The filament is now seen to break up into its definite chromosomes (figs 33–35), and in number these are sometimes easily seen to be the number characteristic for the reduced number, which seems to be eleven for the species in question. Each chromosome, however, is clearly seen, on following its subsequent history, to be bivalent. For the previous parallel arrangement of the threads during the looping-over stage is responsible for the simulation of the duplicate character to be observed in each chromosome at this period. In the most frequently recurring forms, the bivalent chromosomes at this stage resemble double rods, which might easily be mistaken for the shortened and thickened halves resulting from the previously recorded longitudinal fission did not the intervening stages preclude such an explanation. Very often the transverse delimitation give rise to a bent-V-shaped body, the two limbs of which represent a continuous length of the original spirem, and hence clearly betray the bivalent character of the chromosome. It may happen, however, that the halves become entirely separated from each other, and independently of any bending over of the thread. But nevertheless they come together so that the reduced number of (bivalent) chromosomes is affected. In cases such as that just mentioned the conjugation of somatic chromosomes during the heterotype

prophase is placed beyond a doubt. It does not seem to be a matter of any consequence how the bivalent arrangement is produced, since there is so much variability in the process, but the temporary union in pairs of somatic chromosomes is the really important feature.

The further history of the chromosomes is less easily followed than in Osmunda, but the same types are reproduced here in almost every detail, and they pass on to the spindle in a precisely similar manner; perhaps, however, the ring-like figures are rather more frequent in Aneura than in Osmunda.

The spindle in its earlier stages has already been described as a quadripolar structure. The individual kinoplasmic threads can easily be distinguished in good preparations; but as the chromosomes begin to assume their definite form, and before they pass on to the spindle, the quadripolar arrangement becomes obscured, and usually obliterated. The sheaves of fibres become shortened, and hence project less into the lobes, and then the ends fuse in pairs, so that a bipolar arrangement supervenes. But it sometimes happens that a sharp bipolar form is not attained, and then at one or the other end the pole is seen to bifurcate somewhat, in correspondence with its mode of origin.

When they come to lie on the spindle the chromosomes are often difficult to analyse. They may form the twisted figures so frequent in the corresponding stage of a lily, or they may exhibit the form of closed rings with equatorial thickenings, or finally they may form X-like structures (figs. 35, 36). And as the period of the diaster approaches they present the highly characteristic form and arrangement that is met with in the heterotype mitoses of both plants and animals.

When the diaster is formed it is seen that each bivalent chromosome is so divided (fig. 36) that transverse halves (i. e. its monovalent constituents) are distributed to the two daughter nuclei. Sometimes this can be made out very clearly when the ring-like forms break asunder at first at one side. The whole is then straightened out in the direction of

the spindle, recalling the corresponding figures that are so much more frequently to be seen in Tradescantia. But as a general rule the V shape of the daughter chromosome is not easy to identify. They are swollen and stumpy structures, and very seldom show the reopening of the fission that is so conclusively exhibited in Tradescantia and sometimes also in Lilium.

A wall is formed across the interzonal fibres at the close of the heterotype mitosis, and the daughter nuclei at once divide again, the new spindles being formed close together, but their axes not being in the same plane. The fission of these (homotype) chromosomes is clearly longitudinal (Fig. 37), and seems beyond doubt to correspond with the hitherto obliterated primary fission of the spirem thread of the previous karyokinesis.

The four nuclei are thus distributed to the four lobes of the original mother-cell (fig. 38), and the respective lobes are delimited from each other, at the centre of the original cell, by walls that take up the same position as do soap films when placed in boxes of corresponding form. Ultimately fresh walls are formed around the contents of each cell (special mother-cell) and the spores separate by the solution of the original walls. But this process need not be described here, as it is not pertinent to the main objects of this memoir.

IV. Periplaneta Americana.

(*a*) The pre-maiotic period.

As an illustration of the manner in which the sexual cells become matured among the metozoa, no individual type appears to be more suitable, or on the whole more interesting, than the common cockroach.

In this insect, as in so many other cases, the male gland consists of numerous small spaces filled with cells in different stages of development; and as in all cases among the metozoa, these generative cells have themselves arisen through the

continued multiplication of the elements which, in the first instance, constituted the so-called generative blastema of the embryo.

In the adult male, the cells which are about to become sexually mature are found to be still multiplying through the continuation of the same series of pre-maiotic divisions whereby they have been increased from the segmentation of the ovum onwards, and as this pre-maiotic multiplication differs only in certain details from the processes already described so fully in numerous treatises upon cell division in general, it will only be necessary here to briefly recount the successive stages of the process, so that the history may appear complete and the special peculiarities of the somatic cell division in the cockroach may be brought into sufficient prominence.

In the example we have chosen the cells of the pre-maiotic series which are about to divide, whether they are encountered within the sexual glands or elsewhere in the tissues of the body, present the rather characteristic appearance represented in fig. 40, a very irregular network of chromatin and linin being grouped within the nuclear membrane round one or two highly chromatic nucleoli. Among such elements mitosis is ushered in by the increasingly chromatic appearance of the cells, this being followed by the gradual evolution of a definite arrangement of the chromatin, and in the particular type under consideration the latter process is not by any means without interest from a general point of view.

At first the cells which are preparing for division present an almost even granulation of the chromatin within their nuclei, and this in its consistency strongly suggests a foam structure of the ordinary type; but after a time the "chromatic confusion," as it were, sorts itself out into obvious condensations or cloudy areas, and it is apparently unquestionable that each of these primitive chromatic clouds is individually the forerunner of one of the future chromosomes (figs. 41–44).

The gradual condensation which occurs in each such cloud

proceeds, moreover, in such a manner that the chromatic granules become arranged or grouped in two distinct rows, or tracts. So that by the time the individual chromosomes have attained to some sharpness of definition they appear also as if they had been split longitudinally from end to end. In the cockroach, however, it is obvious that this split has not arisen from the sundering of a pre-formed riband, but by the gradual grouping of the chromatin granules into the form of a short double rod (figs. 46–48).[1]

It will have been seen that the method of chromosome formation here depicted presents nothing exactly comparable to the long spirem thread which is figured in so many of the existing accounts of pre-maiotic division which have hitherto appeared.

In all cases which we have examined the number of the rod-like chromosomes which are eventually produced appears to be generally thirty-two; that is, by counting the chromosomes in a large number of cells, and then taking the average of such counts, the number thirty-two has always been attained. But it is not intended, nor should it be assumed that there is an absolute numerical rigidity in all the individual cells; for many figures have been encountered in which the number appeared to be more or less than this, by one, two, or even more, yet in these cases there was no reason to suppose that the cells under examination had in any way been altered by manipulation.

When the pre-maiotic mitosis has reached the above stage the cells which present themselves in groups with the short double chromosomes just described possess the characteristic appearance represented in fig. 47; while about the same time the parts of the karyokinetic figure related to the centrosomes, as well as these bodies themselves, emerge once more into prominence.

All the ensuing stages of the pre-maiotic divisions are in

[1] Cf. Farmer and Shore, "On the Structure and Development of the Somatic and Heterotype Chromosomes of Tradescantia Virginica," 'Quart. Journ. Micr. Soc.,' 1904.

perfect accord with what has hitherto been described, the centrosomes separate to the opposite ends of the cell, where they lie a short distance within the bounding membrane, while at the same time the chromosomes, after being bunched in a confused mass, are gradually drawn into the usual equatorial figure (see fig. 51). During this process, however, the short split rods generally become more curved, and since they are all attached by the middle of this curvature to the spindle fibres, they often present the appearance of sharply defined tetrads, the manner in which this appearance is produced in the type under consideration being, however, at once apparent upon comparison (figs. 47–51). It must be admitted that these tetrad figures occurring in the pre-maiotic divisions of the cockroach are singularly like those described among various arthropods by Häcker and others, but always referred by these authors to the process of reduction, and not to the pre-maiotic stage at all.

In the later stages of the pre-maiotic divisions the halves of each of the thirty-two chromosomes gradually separate and pass away to the poles of the spindle figure, to form the group of chromosomes belonging to each daughter nucleus, and the division of the cells becomes complete.

In the cockroach, as in so many other animals, the remains of the spindle persists for some time as a sort of band connecting the daughter cells together, and this connecting spindle relic may still be encountered during several subsequent divisions of the daughter elements; but there are no intermediate bodies produced quite comparable to those originally described by Flemming in amphibia, and seen subsequently in so many other animal forms.

During pre-maiotic divisions, the conspicuous nucleolus of the cells breaks up and is formed anew within the daughter nuclei, the remains of the old nucleoli passing into the cytoplasm where they disappear.

The divisions of the pre-maiotic elements of the cockroach can be followed with the greatest exactitude and ease in the mature testis of this animal, and for all major details the

mode of procedure here pursued is identical with that encountered among the cells composing the rest of the animal's body; for although it is by no means so easy to follow out the whole cycle of events among the cells composing the ordinary body tissues, a sufficient number of phases of division have been encountered to show that the number of the chromosomes is thirty-two and that the characters of the division of these elements are similar to those of the pre-maiotic series of the testis.

The number of the ordinary pre-maiotic divisions which actually occur in the testis and precede the onset of the reduction process is not easy to ascertain; it is not less than six or eight, and it may possibly be as many as ten to twenty; but whatever the number of these divisions there may actually be, the process of pre-maiotic multiplication in the testis, as in the ovary, sooner or later comes to an end, and is succeeded by the chain of events which results in the reduction of the number of the chromosomes in each cell by one half, and the rendering of the resulting elements ready for sexual conjugation.

(*b*) The Maiotic Phase.

The onset of this singular metamorphosis, the maiotic phase, is first apparent by virtue of an alteration in the resting nuclei which are about to enter upon the change. Such nuclei become obviously more chromatic than those of the pre-maiotic cells, whilst the chromatin network, from being loosely scattered through the nuclear substance, assumes a fine and very even granular appearance, which often suggests the existence of a very closely tangled spireme thread. As time goes on, however, the fine meshwork of chromatin becomes more and more definitely arranged—polarized, in fact. That is to say, it presents strands which run round the nucleus in loops, and these as they develop assume a horseshoe form with their rather pointed ends open, and all are collected together at one side so as to form a distinct pole field in the ordinary sense. It is at this period that the sphere and

centrosomes can be first discerned in the cytoplasm opposite the ends of the emerging chromatic loops.

From the time at which these maiotic cells can be first distinguished they present—unlike the pre-maiotic elements which have anteceded them—a single, distinct, and relatively large nucleolus; and during the onset of the synaptic phase this body becomes stretched out and lengthened as the polarization of the nucleus increases, so that eventually it produces a curious and characteristic appearance represented in figs. 53–56.

In the succeeding phases the polarisation of the chromatic loops becomes at first more complete. Or, in other words, the original chromatic meshwork becomes more and more definitely drawn out into the broad, horseshoe-like structures which are represented in figs. 57–58. At the same time the whole chromatic substance of the nucleus tends to contract away from the nuclear membrane towards the sphere (archoplasm). It is this first contraction figure which has often been spoken of as the synaptic contraction, but as a matter of fact there are in reality two contraction stages, of which the figures represented in figs. 53–67, only illustrate the first.

When the chromatic loops have acquired the definite characters delineated in fig. 57, they begin to open out over the surface of the nucleus, and often become actually thinner, until figures like those represented in figs. 63–66 are frequently obtained. The process of unravelling, however, continues still farther than this, until the nucleus presents a typical coarse spireme irregularly distributed over its surface, as is shown in fig. 66.

At about this stage in the cockroach it is generally possible to observe that the nuclear threadwork is becoming longitudinally split, and the appearance which the cells then present is reproduced in fig. 67, the whole of this phase of the division reaching its maximum in such elements as have been represented in figs. 64–67. In all these later figures the cells present the coarse spirem appearance which is so well known. However, it is not in this stage that the

final transverse breaking up of the spirem thread into chromosomes actually takes place. In the cockroach it is easy to demonstrate, positively, that immediately after this period a second contraction stage ensues.

The coarse spirem thread becomes again polarised, and this second polarisation is carried to a far greater degree than in the first contraction figure, as will be seen on comparing fig. 57 and fig. 72. The whole threadwork is, in fact, at last drawn into short thick loops, which usually radiate from a centre in the manner represented in fig. 69. Nevertheless, at this period it is usually possible to trace the original longitudinal splitting of the threadwork running round the limbs of the individual loops. Or, in other words, the series of figures (67–72) show that the short loops in fig. 72 are not to be taken as portions of the opened-out split in the threadwork represented in Fig. 68, but as divided threads which have become bent round upon themselves.

From the stages represented in figs. 56–60 we pass to such stages as those reproduced in figs. 71–72, in which it can be seen that the loops arising in the second contraction figure are directly metamorphosed into the diaschistic (heterotype) chromosomes; but even in this later stage it is often possible to trace the remains of the original split (the anaschistic fission) running round the edges of the diaschistic (heterotype) loops or rings, as in fig. 73.

From a contemplation of the above facts and figures we are brought to the conclusion that the diaschistic heterotype chromosomes are different in origin and character from those of ordinary pre-maiotic cells. Each of these loops or rings does not represent the opening out of a segment of split thread-work, as Flemming originally conceived, but is in reality seen to be composed of a portion of the split spirem-thread which has become bent round upon itself in the form of a ring or a loop. Moreover, it often happens that the diaschistic chromosomes, instead of assuming the form of a loop or ring, appear as a couple of thick rods placed side by

side, and not attached together at either end. Each rod, however, is longitudinally split, and the pair together constitute a diaschistic (heterotype) chromosome of a characteristic and familiar type.

Now, as is well known, the number of the heterotype diaschistic chromosomes is always half that in the preceding divisions, and in such a diaschistic figure as the above we have a condition of things which would be exactly attained if two ordinary somatic chromosomes were to become associated together.

In many instances, even before the nuclear membrane has disappeared, we have found that the short, thick loops have already divided transversely in their curved portion, thus : (○) and through the existence of such figures we immediately see how those diaschistic (heterotype) chromosomes having the form of a pair of actually, or potentially, split rods have been produced. In the case of the more usually shaped chromosomes, as division proceeds the separation of the loops or rings into two halves takes place while the elements are on the spindle, and is brought about by a similar transverse breaking of the curved loop. Or the process may be still further modified in detail in a number of ways which we have already described in a former paper.[1]

Whatever the exact method adopted the result is the same, and it comes to this: that the pre-maiotic number of chromosomes tends to be formed; that these for a longer or shorter time remain united in pairs, so that there are only half as many chromatic aggregates in the cell as in the case of the ordinary pre-maiotic divisions, while during the later state of the first maiotic or heterotype mitosis the united chromosomes simply separate from one another and pass in their entirety into each of the daughter cells.

In the cockroach there are, as a matter of fact, two chief variations of the manner in which the diaschistic (heterotype) chromosomes are arranged, and separate from one another on the spindle, during the later stages of division. In the one

[1] Farmer and Moore, loc. cit.

we have the chromosomes in the form of small rings which divide in the manner represented in figs. 74, 75; in the other the ring is open at one side, or is a loop, and being attached to the spindle in the fashion shown in fig. 77, opens out in the manner represented. In this latter variation the final condition of the dividing chromosomes is extremely interesting; for the original longitudinal split can be traced with great clearness, and can actually be watched as it forms the characteristic longitudinal split of the daughter chromosomes of the first maiotic (heterotype) division first described by Flemming, in the salamander, among animals, and by Strasburger, in Tradescantia, among plants. From such figures in the cockroach it becomes at once obvious that this singular and well-known split condition of the daughter chromosomes of the first maiotic (heterotype) division, to which the above authors long since drew attention without offering any explanation, is nothing more nor less than the persistence in these daughter elements of the original longitudinal split of the synaptic spirem thread.

From the above it will have become obvious that in the cockroach the first maiotic (heterotype) division differs from the pre-maiotic divisions which have anteceded it in this; that here, instead of the chromosomes consisting of thirty-two split rods or lengths of the spirem thread the halves of which will be distributed between the daughter cells, we find that the spirem thread-work tends at first to separate into only half as many lengths, that eventually the full somatic number of elements are formed, but these remain associated together in parts to form the potentially double heterotype chromosomes; or, in other words, the first maiotic division is distinguished from the pre-maiotic divisions by the temporary union of the pre-maiotic chromosomes in pairs, and by the simple separation of these elements during the ensuing mitosis. In this way the cells of the second maiotic generation receive only half the number of chromosomes which have characterised the preceding generations. Nevertheless, in the diaschistic (heterotype) prophase the thread-work is longitudinally split,

just as it is in the pre maiotic divisions, and it is this splitting in the segments of the chromosomes which constitutes the longitudinal fission seen in the daughter elements as they recede from one another.

In the cockroach after the first maiotic (heterotype) division has been completed the resulting nuclei pass into a condition of almost complete rest. That is to say, the nuclei again return to the state in which there is merely a coarse chromatic reticulum where it is impossible to trace the daughter chromosomes any further, and it is consequently only after a considerable period that the second maiotic (homotype) division is brought about. In this (the last division of the series), as in the ordinary pre-maiotic divisions, the sixteen chromosomes emerge each from definite chromatic condensations, wherein the chromatin becomes again arranged in two thick streaks or bands, the chromosomes presenting the appearance of so many short split rods; and as division proceeds these pass on to the spindle and divide in the ordinary pre-maiotic manner.

Thus, although it would seem to be strongly suggested that the ordinary longitudinal split of the segments in the synaptic spirem thread constitutes the fission by means of which the reduced number of chromosomes in the second maiotic mitosis are ultimately divided, this is not absolutely demonstrated in the Periplaneta itself.

V. Elasmobranchs.

(*a*) The pre-maiotic phase.

In view of the remarkable character of the reduction process as it appears to be carried out in the typical arthropod example constituted by the cockroach, we have re-examined the elasmobranch material which had been obtained and already described by one of us [1] in 1894; such a re-examina-

[1] Moore, J. E. S., "On the Structural Changes in the Reproductive Cells during the Spermatogenesis of Elasmobranchs," 'Quart. Journ. Micr. Sci.,' vol. 38, new series.

tion has made it obvious that although the main features of the spermatogenesis of these fishes were correctly ascertained, certain aspects of the maiotic phase were not fully appreciated at the time.

In many ways the functional male gland of an elasmobranch is an admirable object for the study of all the stages of development in the sexual cells; but it is also true that as far as the heterotype prophases are concerned, the phenomena in these fishes are somewhat confusing, and are far more readily interpreted correctly, after a knowledge of what actually takes place has been obtained in some form like that of the cockroach.

In the various forms of elasmobranch testis the young tubules are found crowded with cells which are just rapidly multiplying through successive pre-maiotic mitoses as they do in the testis of the cockroach, the chief distinction between the fish and the insect being that in the former there is present a much more complete spirem thread than in the latter; in fact, we have here pre-maiotic prophases which are directly comparable with those already fully described by Flemming and others in several amphibian types.

A long coiled threadwork is ultimately formed which splits longitudinally and then breaks up into lengths, the resulting split segments representing the twenty-four somatic chromosomes. As the mode of division of these cells has been fully figured and described by us, it will be unnecessary to recapitulate the entire sequence here, and we may pass on to a consideration of the first maiotic prophase itself.

(*b*) The Maiotic Phase.

As in the cockroach, cells which are about to pass out of the pre-maiotic cycle and enter upon the synaptic metamorphosis present an increase in their chromatin, and a gradual enlargement, which for a time seems to keep pace with the nuclear metamorphosis. In torpedo and other examples of elasmobranch fishes we find that the very fine spirem

which at first emerges from the resting nucleus gradually becomes, as in the cockroach, more and more polarised; and, just as in the insect, we have found that the subsequent metamorphosis consists of a gradual thickening of the individual threads and an unfolding of the contraction figure into a coarse spirem which in its fully-developed condition is evenly distributed over the surface of the nucleus. At about this period many of the individual threads can be seen to be longitudinally split, and the cells then remain for a long period in the same condition, the threadwork merely becoming thicker and more chromatic as time goes on. When this period has come to an end, as in the cockroach, the threads become once more polarised, and this contraction corresponds with the second synaptic figure previously described. We have found, moreover, that in the elasmobranch as in the cockroach, these secondary loops are unquestionably to be regarded as the individual forerunners of the diaschistic (heterotype) chromosomes; their sides present an obvious longitudinal split, and in many cases the loops become twisted upon themselves as they do in plants; in fact, all the various types of diaschistic (heterotype) chromosomes are found to which we have already referred.

Now, in the amphibia which had been described before we had examined the elasmobranchs spermato-genesis the hollow of the heterotype loop. The aperture in the ring, or the space between the twisted rods with open ends, had always been regarded by Flemming, Meves, and others as the opened-out portions of the original longitudinal split traversing the spirem thread; but when that which happens in the cockroach is borne in mind, it becomes obvious that all the stages in the insect and the fishes up to this point correspond, and consequently it became at once suggested to us that probably these and the subsequent stages among the vertebrates had been misinterpreted.

A careful review of the ensuing stages among elasmobranchs has convinced us that this supposition is correct; and that for all practical purposes the later stages in the first maiotic

(heterotype) division in these fishes are, like the earlier ones, carried out in the same manner as in the cockroach itself. There seems to be no room left for doubt that the coarse spirem contracts again into a polarised figure and that the loops of this second contraction are converted directly into the diaschistic heterotype chromosomes.

We have found no figures which in any way militate against this view of the origin of the heterotype chromosomes among these fishes; and the apparent reason why the process has not hitherto been apprehended seems to be that among elasmobranchs the second contraction-figure, or synapsis, is much more rapid than in the cockroach. Consequently one is apt to pass over its existence, from stages corresponding to that represented in fig. 68 to the later stage given in fig. 73, whereby it might be natural to conclude that the heterotype loop, or ring, arose from the opening out of the longitudinal split in the spirem segments. So far, then, as the origin of the reduced number of heterotype chromosomes is concerned, we reach, after a renewed study of the process in elasmobranchs, exactly the same conclusion as we did in the case of the cockroach; that is, the synaptic and pre-maiotic prophases in the origin of the reproductive elements in these widely separated animal types are apparently identical. In both, the reduction of the number of chromosomes is brought about by a special prophase, wherein pairs of longitudinally split somatic chromosomes become temporarily united together, and afterwards merely separate from one another during the diaschistic (heterotype) division.

In Elasmobranchs the later phases of the first maiotic mitosis have already been fully described by one of us,[1] and at the present time we have nothing to add to the description already published. With respect to the second maiotic division, however, it is now necessary to append some correction to the previous description. In this it may be remembered that the second maiotic or homotype division was described as having the same characters as the first

[1] J. E. S. Moore, loc. cit.

maiotic division itself, or as being a second diaschistic (heterotype) mitosis. This we have found now not to be the case; for although the details in the second maiotic division in these fishes are extremely difficult to elucidate, we have been able, through a careful re-examination, to determine that the apparent similarity of the phases in this to the first maiotic series is fictitious, and that in reality this division has the ordinary pre-maiotic anaschistic characters as in other animals and plants.

We have now dealt fully with a typical insect, and several Elasmobranch types, and the intention has been to use these as illustrations of the manner in which reproductive elements become matured in widely sundered classes of animal forms. It has been found that so far as these different examples are concerned there is a complete parallelism among them all. It has been shown further that the similarity which exists between the reduction in insects and Elasmobranchs also subsists between all these zoological examples and the various vegetable forms previously described. Throughout the whole series the process is carried out on an essentially similar plan. In themselves, and certainly when we bear in mind what has already been ascertained with respect to a host of other animal and vegetable forms, the present examples would be quite sufficient to indicate that there exists throughout the whole range of living forms a fundamental similarity in the manner in which the numerical reduction of the chromosomes is achieved. Still, it will also be apparent that, especially among the vertebrate class, several amphibia and mammals have been dealt with by various authors in great detail, notably salamander, triton, and the rat, and it will also be apparent that the results attained in relation to these are not in accord with those put forward with respect to insects and fishes by ourselves. Especially in the able works of Flemming and Meves, we find a view taken with respect to the origin of the diaschistic (heterotype) chromosomes similar to that held by many botanists with respect to the flowering plants—

namely, that the loops and rings arise through the opening out of the longitudinal split in the segments of the spirem thread. A careful re examination of our own amphibian material has, however, convinced us that the older interpretation of the origin of the diaschistic (heterotype) chromosomes is, in this respect, incorrect. It would seem, indeed, that amphibia, although possessing gigantic cells, are peculiarly unfavourable objects for the elucidation of the prophases of the first maiotic division. But when re-examined after a knowledge of what occurs in the corresponding stages among the more favourable materials presented by many plant and some animal forms, we have been irresistibly driven to the conclusion that the rings in the amphibia, like those of the cockroach, are produced by a folding or some other form of association between two portions of the split chromatin riband. It is quite easy in the case of axolotl and triton to discern the longitudinal split in many fully formed diaschistic (heterotype) loops, and in these forms we find no essential difference between the particular phases of the first maiotic division and what occurs in a more obvious manner among the types we have previously described.

It remains, then, merely to refer briefly to what is known with respect to this process in the higher vertebrates, such as the birds, reptiles, and mammals. Of the first two we have at present little to say; but with respect to mammals, we have examined the prophases of the first maiotic division in the testis both of the mouse and the rat,[1] with the result that we have become assured that the evolution of the diaschistic (heterotype) chromosomes is here the same as in the lower forms. Quite recently we had the opportunity of examining the same stages in man; and although it is necessary that the full results of this investigation shall be published in a separate memoir, it may be stated that with respect to the prophases of the first maiotic (heterotype) division, and the manner in which the diaschistic chromosomes are evolved,

[1] Moore, J. E. S., "Some Points in the Spermatogenesis of Mammalia," 'Int. Monat. f. Anat. u. Phys.,' 1894.

our results in the case of the human species are identical with those obtained among the lower members of the vertebrate class.

Conclusions.

In attempting to form any opinion respecting the conclusions which may naturally emerge from the preceding mass of details respecting maiosis, or the reduction of the chromosomes, in animals and plants, it will have become evident, as was pointed out in the introductory portion of this memoir, that whatever particular significance we may be inclined to attach to the process in question, the essential details are in all respects similar throughout the higher numbers of both the animal and the vegetable kingdoms.

Or, in other words, it follows that whatever significance may ultimately be attached to maiosis itself, this process is probably one of the most fundamental facts with which biologists will have to reckon. Such being the case, it may not be undesirable briefly to review the essential features of reduction before attempting to draw whatever conclusions may seem legitimate from the facts that have now been ascertained.

In all multicellular animals and plants, the elements which from the first division of the ovum onwards gradually build up the soma or body of such an organism multiply in general by the process of karyokinesis, and in all cases this somatic cell division is carried out on an essentially similar plan. In the better known examples of such division, like the types described by Flemming, Rabl, Strasburger, and many others, the obscure chromatic reticulum of the resting nucleus is transformed into an increasingly definite spirem thread, which, when fully formed, often presents the appearance of a single and endlessly coiled filament. It is this thread which ultimately breaks up into the number of segments that are destined to constitute the future definitive chromosomes. Yet although this interpretation could be put upon the appearances observed during the prophases of division in a large number of animal and vegetable forms, there certainly exist

other instances from which it is more natural to draw a somewhat different inference. In the cockroach, for example, the chromosomes of the pre-maiotic mitoses do not originate through the breaking up of a coiled spirem filament. For in this example it is usually possible to see the limits of the individual chromosome even when the nucleus is in a condition indistinguishable from the rest. The primordia (or "anlagen") of each future chromatic element first become discernible in the form of a slight chromatic condensation. At such a time the linin masses which will be involved in the future chromosomes appear always to be visibly discrete and separated from one another. The more or less completely resting aspect of the cell is produced by the linin framework of each chromosome possessing an alveolar or reticular structure in which the chromatin is irregularly distributed. The evolution of the chromosomes is brought about by the separation and condensation of each vesicular linin element, and the chromatin granules become eventually closely packed together within the axis of each condensing element. A somewhat similar state of affairs has been observed in the somatic prophases in Drosera and in Tradescantia, and in all these instances it would seem that it is not strictly accurate to assume that the chromosomes originate through the breaking up of a spirem filament; for in none of them is the spirem, as generally understood, produced in the first instance, and in Blatta it is never formed at all.

The facts revealed by the above instances are not without theoretical importance. They strongly favour the hypothesis of the persistent identity of the chromosomes from generation to generation, and it is not impossible that they show more clearly than the commoner types of cells the manner in which the chromatic elements become obscured during rest and reappear at each succeeding divisional prophase. For example, when, as usually is the case, the somatic chromosomes are relatively very long, thin, rod-like structures, if these persist as vesiculated masses within the resting cell their existence would not generally be evident owing to the dis-

tribution of the chromatin throughout the vesiculated linin. Indeed, as such cases as those represented in figs. 42–46 will show that, when the linin masses begin to separate and contract, and the chromatin collects along the axis of each originally vesiculate filament, there will result the appearance of an endless spirem which has generally been described.

However, by whatever method chromatin thread-work actually originates, it generally does appear later as an intricate coiled mass of chromatic filaments, and these filaments eventually separate out and contract into the characteristic number of rod-like somatic chromosomes.

In the somatic (anaschistic) divisions by means of which the tissues of an adult multicellular organism are gradually built up this late nuclear spirem sometimes, but by no means always, shows indications of being longitudinally split before the somatic chromosomes are definitely separated out. But at whatever time the longitudinal fission first becomes actually apparent, it is always to be seen when the chromosomes are finally grouped upon the spindle in the so-called equatorial plane; and this splitting invariably provides the mechanism by which the halves of the somatic (anaschistic) chromosomes are distributed in equal numbers among the daughter nuclei of each succeeding generation of cells.

The sharp distinctions between the innumerable somatic (anaschistic) divisions which follow one another during the ontogeny of a multicellular organism and the single heterotype (diaschistic) mitosis whereby certain cells of the body are ultimately fitted for sexual union, is brought about by the intercalation of a series of definite changes which are characteristic of the prophase of this particular division. In their entirety, these added portions of the metamorphosis constitute what has already been distinguished by us as the synaptic change, and the whole process of synapsis consists essentially of the following successive phases:

Whilst at first they are indistinguishable from the resting or pre-maiotic cells, those which are destined to proceed to the heterotype mitosis become at first characterised by the closer,

more chromatic, and often polarised, arrangement of their nuclear reticulum. In the more readily elucidated examples, such as Blatta or Osmunda, this polarisation increases and the chromatin becomes finally arranged in a number of definite loops. In a large number of instances these loops can readily be counted, and when this is the case, there are always found to be half as many loops as there were somatic chromosomes in the preceding pre-maiotic divisions.

At the same time, the whole chromatic network contracts away from the nuclear membrane, this change producing the First Contraction figure. As time goes on the loops become not only increasingly chromatic but also opened out again, until the apparent polarisation is more or less completely lost and the nuclei present the well-known coarse spirem figure within the strands of which double beading or actual longitudinal fission is nearly always more or less apparent. The coarse spirem figure often constitutes a prolonged phase, but it is in all cases ultimately succeeded by a short-lived and easily missed resumption on the part of the split chromatic thread-work of its earlier polarised arrangement; and this is followed by a strong Second Contraction and thickening of the individual loops. Even before the second contraction has fully supervened, the longitudinal fission of the thread-work has in the great majority of cases almost closed up and disappeared; and although the exact details of the subsequent evolution may, and to some extent do, vary in the different types, the general statement that each of the individual loops in this second contraction figure becomes directly converted into one of the heterotype chromosomes sufficiently expresses the really essential parts of the process.

In some cases, as in the cockroach and Osmunda, the loops, throughout the whole series of events, remain distinct from one another, with their free ends open in the region of the pole field; but during the later stages of their formation they often break transversely in the curve of the loop as well. Consequently since the number of unbroken loops is half the number of pre-maiotic chromosomes, in this stage as well

as much later we reach, in the prophase of the heterotype mitosis, a condition of things wherein the full number of the pre-maiotic chromosomes are really separated out, although disguised by the fact that the pair represented by a disjointed loop always remain associated. The different forms which this association may take give rise, as we have seen, to all sorts of different figures; thus, in the later stages of the division we sometimes encounter heterotype chromosomes having the appearance of loops twisted upon themselves; or again, we may have a pair of rods open at both ends, joined at both ends in the form of a ring, or lying over one another at right angles in the form of a cross. But in whatever form the heterotype chromosomes appear they are always obviously to be interpreted as pairs of somatic rods attached or associated together.

In the succeeding stages of the division, when the chromosomes are definitely attached to the spindle, the individual somatic elements of which each chromosome is composed become simply separated from one another and pass into the daughter cells. And in some cases, as in the cockroach, while this separation is in progress, and the chromosomes become lengthened out upon the spindle, the original longitudinal split in each is again quite clearly evident. Such figures explain at once the real meaning of the longitudinal fission which has frequently been observed in the daughter elements as they divaricate from one another during the heterotype diaster.

The second maiotic (or homotype) mitosis follows immediately upon the heterotype (first maiotic) division. In the cases studied by us it consists clearly in the completion of that initial longitudinal fission of the spirem that was accepted (but not finished) during the prophase of the first of the two maiotic divisions.

Thus the essential peculiarities of the maiotic phase can be explained as follows: They are due to the coherence in pairs of pre-maiotic chromosomes and to the intercalation of a special form of chromosome-distribu-

tion during the course of what otherwise would not differ materially from an ordinary pre-maiotic mitosis. In the first of the two divisions, a distribution of entire pre-maiotic chromosomes is secured, and thus the number of these bodies is really halved. In the second division, the longitudinal fission begun, but temporarily arrested, in the preceding prophase takes effect. Consequently this mitosis as a whole resembles the later stages of an ordinary one save in the reduced number of the chromosomes.

It is, of course, possible that the succession of these two series of events might become inverted, and cases have been described in which the first (heterotype) maiotic division is said to be anaschistic, while the second one is diaschistic; but if fresh investigations should confirm this, it would in no way detract from the utility of regarding the collection of events in question as constituting a definite and essential phase (maiosis) in the cellular life history of an organism.

It is obvious from the foregoing description of the events characteristic of maiosis that in any succeeding cell-generations we shall encounter only half the number of chromosomes that were present before that phase supervened; and it can only be after fertilisation, or some other process analogous to that described by us for the case of apogamous ferns, that the reduced (post-maiotic) number can again be brought back to the full pre-maiotic complement. The number of post-maiotic cell-generations varies. There may be none, as in the normal cellular cycle of an animal, in which the differentiation of sexual cells follows immediately upon the second maiotic (homotype) division. On the other hand, there may be a considerable number, as for example in ferns, in which the whole prothallial individual consists of post-maiotic cells. In animals it is only in certain pathological growths that an analogous condition appears to obtain.

In plants, however, there is no case known at present in which the maiotic phase leads directly to the production of sexual cells, although in Fucus, and also in some of the

highest flowering plants, the formation of the ovum (oosphere) is only separated from it by a single mitosis. In these cases it appears probable that the small number has been brought about by a process of shortening the life history, and it is probably correct to say, for the great majority of plants at any rate, that the occurrence of a number of post-maiotic cell-generations is the rule.

The facts of reduction, as set forth in this paper, appear to afford strong grounds for supposing the chromosomes to be permanent structures that retain their identity from one generation to another in the individuals composing a species. This aspect of the question has already been touched on in the Introduction, but its importance is so great that a few additional remarks are called for here.

We have seen that, notwithstanding the impossibility of recognising the delimitation of a chromosome in the resting nucleus, the chromatin or nuclein nevertheless does aggregate in the linin, at the very commencement of prophase, in such a way that it is difficult to escape the inference that the reconstitution of the chromosome represents, mutatis mutandis, the exact converse of the series of changes witnessed during the preceding telophase. Many other authors have been driven to a similar conclusion, and we think that in favourable instances, such as those of Tradescantia and Periplaneta, the evidence in support of the view that would regard the whole process as an unravelling, rather than as a new construction, is extremely cogent. The close correspondence between the actual primordia of the chromosomes, before the spirem thread is built up, and the vacuolating chromosomes of the telophase can hardly be accidental, and, moreover, the evidence based on the identity in numbers cannot be disregarded.

Again, the nature of a reduction which resolves itself into a sorting of chromosomes rather than that of a mere halving of chromatic substance is not easy to explain apart from the existence of a specific individuality that is vested in each one of the structures in question.

And finally, the reappearance during a long series of divisions of chromosomes that can be recognised by some peculiarity such as that of size, as in the case of Brachystola mentioned by Sutton,[1] as well as the remarkable features to be observed during the heterotype mitosis of Drosera hybrids described by Rosenberg,[2] appear only to find a satisfactory explanation on the assumption of persistent identity.

It will be remembered that Rosenberg found that in Drosera rotundifolia there were twenty, in D. longifolia ten, chromosomes during maiosis. Consequently, in the hybrid forms there were normally thirty in each somatic nucleus. When reduction supervened, it might have been anticipated that fifteen would have been the number produced. Instead of this, Rosenberg found in every case that twenty were present. But of the twenty, ten were large and ten were small; and the inference drawn by him was that the ten large ones were bivalent, resulting from the union of pairs derived respectively from D. longifolia and D. rotundifolia, whilst the ten small ones represented single chromosomes that originated from the surplus number (ten) of chromosomes belonging to the rotundifolia parent.

It is, however, equally clear that a change, probably of the nature of re-arrangement, may at least occasionally occur in both a plant and an animal. For whilst there is a striking degree of constancy manifested in the number of the chromosomes characteristic of a species, it by no means follows that closely related species possess closely related numbers, such as multiples of one another.

The various species of lilies or of Ascaris afford examples of the truth of this statement. Possibly the alteration in the number of chromosomes may be correlated with an alteration of specific characters such as bring about what De Vries has termed "mutations." But be this as it may, it is clear that

[1] W. S. Sutton, "On the Morphology of the Chromosome group in Brachystola magna," 'Biol. Bull.,' iv.

[1] Rosenberg, 'Ber. Deutsche Bot. Gesellsch.,' 1904.

the chromosomes are variable or constant in something the same way as are the specific characters themselves.

Perhaps we may be permitted to push the matter further. There is a belief shared by some investigators that a very close relation, of a casual nature, exists between the chromosomes, or combinations of chromosomes, and the specific characters manifested by an organism. At any rate, such a connection is demonstrably existent between the nuclei and such characters as is shown, for example, by the character of the larvæ resulting from the fertilisation of enucleated fragments of eggs, by sperms of other species, or even genera, of Echinoderms.[1]

And the remarkable monstrosities, and, still more, the occasional normal larvæ, produced after polyspermy find their most natural explanation in the view that the main direction of the course of ontogeny is to be attributed to the chromosomes. The analysis made by Boveri [2] of the chromosome distribution to the blastomeres of eggs which had been fertilised by two sperms showed strong reasons for concluding that the peculiarities in the resulting offspring are due to disturbances in the normal relations of chromosomes in the cells to which they are distributed. For polyspermy is usually followed by pluripolar mitoses; and by observing these and then separating the first-formed blastomeres it was possible to make a comparative study of the deviations from the normal form under different conditions of chromosome distribution. Thus it is clear that in a tripolar mitosis there is some chance that two, or at least one, of the three

[1] The parthenogenetically produced echinoderm larvæ are specially interesting in this connection since they only contain half the somatic number of chromosomes, since they result from the asexual development of a post maiotic cell (ovum or sperm). This fact proves that the group of chromosomes present in such a post maiotic cell are sufficient to ensure correct development, and, taken with the circumstances detailed in the following paragraph, lend support to the view that maiosis leads to the separation of the male and female halves that are temporarily united in the heterotype prophase.

[2] Boveri, 'Ergebnisse ü. d. Constitution d. Chromatischen Substanz des Zellkerns,' Jena, 1904.

resulting cells might receive the entire lot of chromosomes contributed by one of the three gametes that have taken part in the previous fusion. In such a case a normal larva might result. On the other hand, with a quadripolar mitosis such a sorting would be almost impossible. Boveri found that the facts accorded well with the hypothesis, and hence concluded that the normal characters of the larvæ were dependent on the appropriate distribution of the chromosomes.

The very remarkable results obtained from crossing hybrids are also found to accord very well with the view of the chromosomes regarded as persisting individuals, although, of course, such results could be equally well accounted for on the supposition that there exist other physical entities to which the manifestation of specific or individual characters could be ascribed, provided they could be shown to persist and to be equally distributed in the same way as we now know the chromosomes to be. But failing their demonstration, we may reasonably admit the claims of the chromosomes to represent the physical machinery to the operation of which the manifestation of the characters in question is to be ascribed.

We would, however, reiterate here the reservation made by us already. We do not look on the chromosomes as primordia of characters, but as agents, by the influence of which on the rest of the protoplasm are incepted those complex physical and chemical changes that culminate in the production of the individual characters.

Too many cases are now known to conform to the Mendelian rules when hybrids of the first (and succeeding) generations are interbred with each other for the results to be a mere matter of chance; and they point strongly in the direction of the existence of a structural, rather than a purely dynamical, combination as responsible for the phenomena in question.

Assuming the causes responsible for the characters or groups of characters concerned to be resident in the chromo-

somes, it is clear that on the basis of the mode of reduction maintained in this paper the Mendelian proportion of $D + 2DR + R$ ought to follow, where D represents a character (dominant) and R the correlative character (recessive) derived from each parent respectively. It is further obvious that such a result can only follow provided the chromosomes of the one parent combine with those of the other in such a way that each bivalent chromosome of the heterotype prophase consists of somatic chromosomes derived from each of the two parents respectively. For if such bivalent chromosomes are formed of pairs derived from the same parent, then a simple analysis will show that quite different relations will obtain, and that in the case of the further hybrid offspring the D and R qualities will not be present in equivalent proportions. The latter case would, however, not affect the validity of the views here advanced of the nature of reduction, but only the parental origin of the constituents of each bivalent chromosome.[1]

Of course the simple Mendelian relation will only occur in cases in which the chromosomes are distributed in the average manner. If some combinations are more favoured than others, then the proportions will be correspondingly disturbed. Similarly with the mosaic hybrids; these might be due, as has been pointed out by others, to a preponderant influence of certain chromosomes, or of combinations of each, in certain parts of the organism. But we would suggest that it might also be explained in another way. The chromosomes, as we have been careful to point out, cannot be regarded as the primordia of characters, but only as the agents that are competent to produce serial changes in the protoplasm they can influence. This implies that the substance on which they work, or which they can "activate," must also be reckoned with. The recent work on regeneration clearly emphasises the importance of the cytoplasm, which in this connection may be compared with raw material, and it is certainly a factor by no means destitute of significance. If the raw material differs,

[1] See footnote on p. 551.

then the result cannot be expected to be identical, and in the interaction of the chromosomes on the one hand, and the cytoplasm on the other, we may perhaps find a clue to the explanation of the sudden sports and other variations often met with in hybrids. For these might seem at first sight to be antagonistic to any form of explanation primarily applicable to the simpler Mendelian cases. But this aspect of the matter is clearly a matter for experimental investigation, and we have only introduced it in this place because we think that the conclusions to be drawn from the behaviour of the nuclei during the reduction divisions suggest that such experiments would probably be fruitful in results.

We take this opportunity of expressing our sense of Professor Ray Lankester's kindness in discussing with us the questions of terminology which have arisen in connection with the subject of this memoir.

EXPLANATION OF PLATES 34—41.

Illustrating Prof. J. Bretland Farmer's and Mr. J. E. S. Moore's paper "On the Maiotic Phase (Reduction Divisions) in Animals and Plants."

The figures 1—38 inclusive refer to the divisions of the spore-mother-cells, and all except Figs. 20, 21, 37, and 38 refer to the first (heterotype) mitosis.

The Figures 40—87 refer to the somatic, heterotype, and homotype mitoses of Periplaneta Americana.

Figs. 1—21.—Lilium candidum.
Figs. 22—28.—Osmunda regalis.
Figs. 29, 30.—Psilotum triquetrum.
Figs. 31—38.—Aneura pinguis.
Fig. 39.—Diagrams illustrating various interpretations of the reduction phenomena.
Figs. 40—52.—Periplaneta Americana, somatic mitosis.
Figs. 53—79.— ,, heterotype mitosis.
Figs. 80—87.— ,, homotype mitosis.

FIG. 1.—Very early stage of prophase of first maiotic (heterotype) mitosis, first appearance of chromatic fibrils.

FIG. 2.—Slightly later stage, first contraction figure.

FIG. 3.—The opening out of spirem after the first contraction. The beginning of longitudinal fission is shown.

FIG. 4.—Slightly later stage, longitudinal fission completed.

FIG. 5.—The divarication of the longitudinal halves of the spirem. The second contraction figure is just commencing.

FIGS. 6, 7, 8, 9, 10.—Stages in the second contraction (synapsis). The longitudinal fission still clear, but the thread as a whole is contracting and thickening.

FIG. 11.—Still later stage, showing the loops and parallel arrangement of the spirem, with indications of the fission now almost obliterated.

FIGS. 12, 13.—The chromosomes rapidly forming, indications (clear in one of fission still apparent.

FIG. 14.—The congregation of the chromosomes just before the formation of the equatorial plate.

FIG. 15.—The chromosomes on the equatorial plate, illustrating some of the various shapes commonly present.

FIGS. 16, 17.—Diaster (heterotype). The chromosomes show the re-appearance of the longitudinal fission. The variation depends on the modes in which they are arranged on the spindle.

FIG. 18.—Late anaphase of diaster (polar view).

FIG. 19.—Telophase of heterotype.

FIG. 20.—Homotype diaster.

FIG. 21.—Homotype telophase.

FIG. 22.—Early spirem of Osmunda.

FIG. 23.—Late spirem, showing the longitudinal fission and the looping of the spirem.

FIGS. 24, 25.—Later stages, the chromosomes definitely isolated.

FIGS. 26, 27.—The various forms assumed by the chromosomes during their later differentiation.

FIG. 28.—The heterotype equatorial plate stage of Osmunda.

FIG. 29.—Psilotum, early stage in development of the heterotype chromosomes, after the spirem phase is over.

FIG. 30.—Psilotum, heterotype chromosomes corresponding to those of Osmunda in fig. 27.

FIG. 31.—Aneura pinguis. Early prophase. In each of the cells shown a centrosphere is figured.

Fig. 32.—The spirem thread is split.

Fig. 33.—The chromosomes are delimited, longitudinal fission obvious in some of them.

Figs. 34, 35.—Chromosomes contracting to their definite form. The quadripolar achromatic figure visible in Figs. 31—35, especially in Fig. 34.

Fig. 36.—The equatorial plate stage.

Fig. 37.—The homotype mitosis, equatorial plate stage, one spindle in profile, one in polar, view.

Fig. 38.—Anaphase of the homotype mitoses.

PERIPLANETA.

Fig. 40.—Resting pre-maiotic cell from the testis.

Figs. 41—44.—Early stages in the formation of the pre-maiotic chromosomes.

Figs. 45—48.—Still later stages in the formation of the pre-maiotic chromosomes.

Figs. 49, 50.—Early stages in the formation of the pre-maiotic spindle figure.

Fig. 51.—Cell in the "equatorial plate" stage.

Fig. 52.—Cell showing the separation of the daughter chromosomes in an ordinary pre-maiotic division.

Fig. 53.—Cell in a very early stage of the first maiotic (heterotype) prophase.

Fig. 54.—Cell a little later, showing the polarisation of the chromatin, and the first contraction of the chromatin from the nuclear wall.

Fig. 55.—A later stage in which both the polarisation and contraction is more strongly marked.

Fig. 56.—A cell in which the chromatic loops characteristic of the heterotype division have become differentiated.

Fig. 57.—A later stage during the differentiation of the loops. In this cell the contraction of the chromatin has about reached its maximum.

Fig. 58.—Cell in which the loops are beginning to open out again.

Figs. 59, 60.—Stages in this process.

Figs. 61—63.—Still later stages, in which the loops are still further opened out to form the coarse spirem, and in which the longitudinal fission of the thread-work is becoming visible.

Figs. 64—66.—Coarse spirem stages.

Fig. 67.—Later stage, in which the thread is again becoming polarised, and in which the fission is well seen.

FIG. 68.—A cell in about the same stage as in fig. 67.

FIG. 69.—Cell in still later stage, where the second or "synaptic contraction" is fully formed and the fission of the thread is no longer visible.

FIG. 70.—The same stage, another view.

FIG. 71.—A cell in which the loops are becoming still more thick and contracted.

FIG. 72.—Cell showing the condition of the loops, at the time of the first appearance of the heterotype spindle figure.

FIG. 73.—Cell in which the spindle figure has reached a later stage and in which the loops which now constitute the heterotype chromosomes have become separated. In one the original split is still, however, clearly visible.

FIG. 74.—Cell showing the manner in which the heterotype chromosomes may be arranged in the equatorial plate stage.

FIG. 75.—A later view of the same.

FIG. 76.—Still later stage in the heterotype division.

FIG. 77.—Slightly earlier stage with the heterotype loops spread out on the spindle, and their separating halves showing the longitudinal fission as they divide.

FIG. 78.—Reconstruction of the nuclei after the heterotype division

FIG. 79.—Later stages in the same.

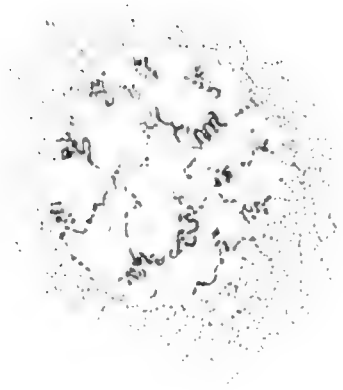
FIG. 80.—Resting cell after the heterotype division and before the homotype.

FIGS. 81—83.—Stages in the formation of the homotype chromosomes.

FIG. 84.—First appearance of the homotype spindle figure.

FIG. 85.—Equatorial plate stage of the homotype division.

FIGS. 86, 87.—Later stages of the homotype division.



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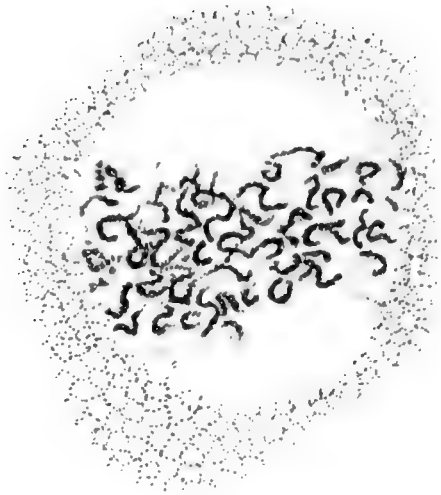

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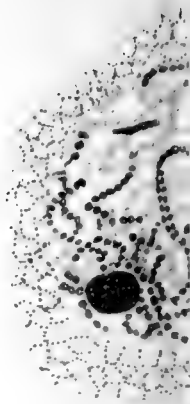

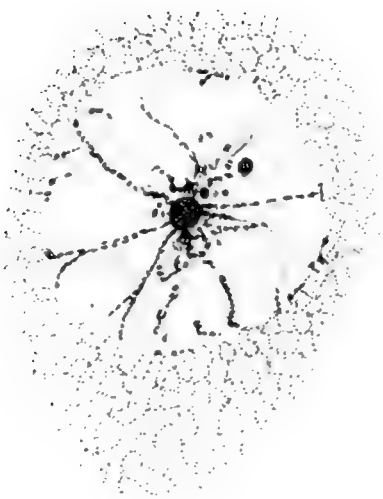

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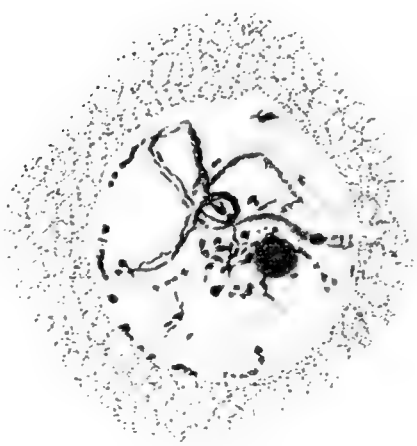

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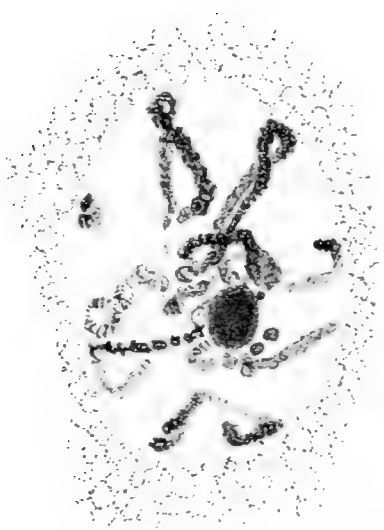

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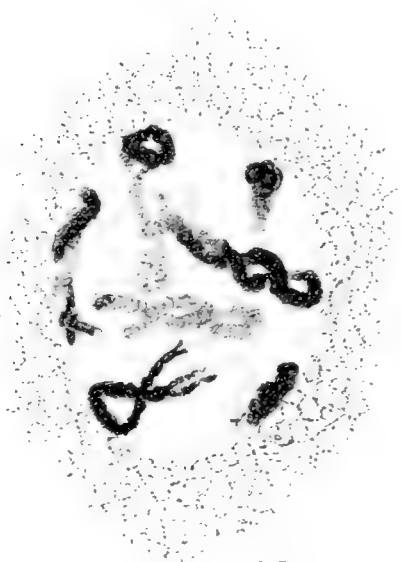

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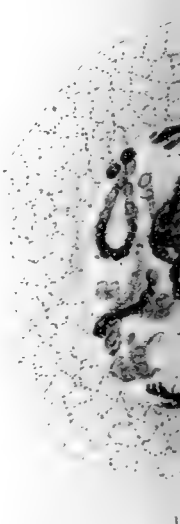

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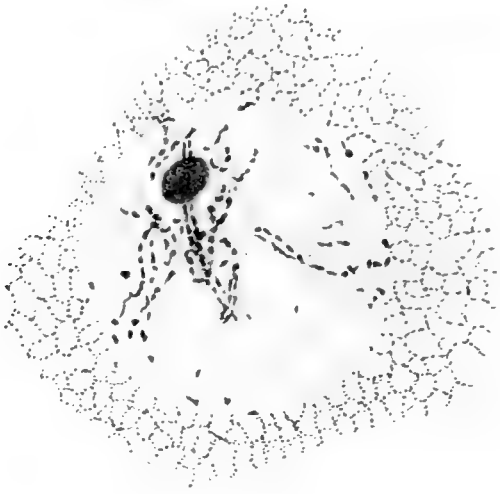

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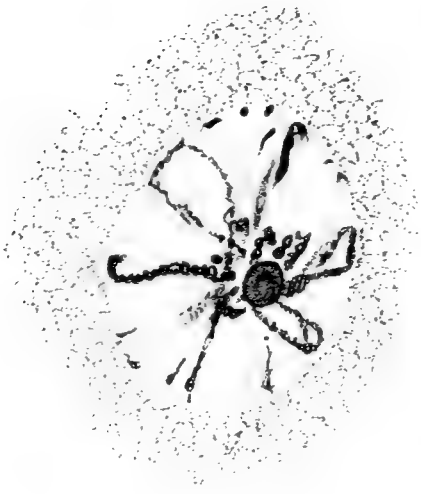

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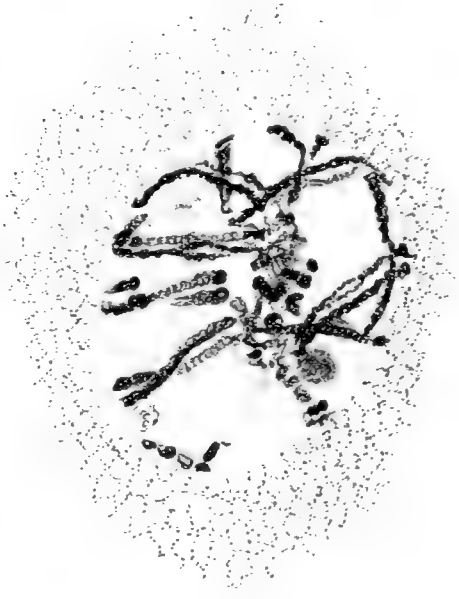

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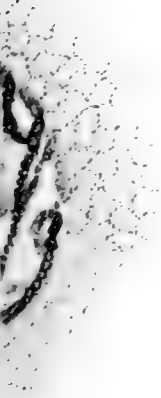

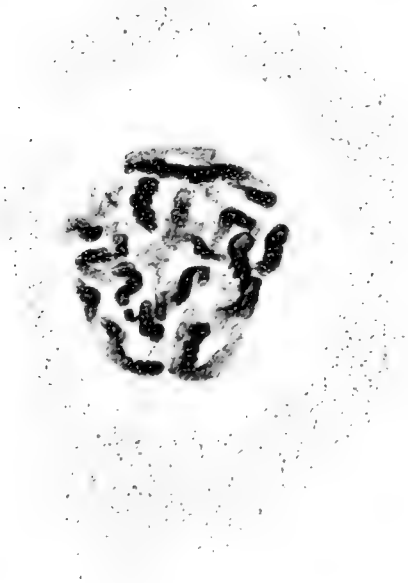

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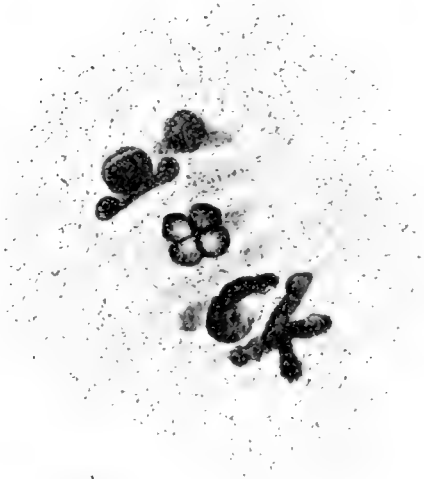

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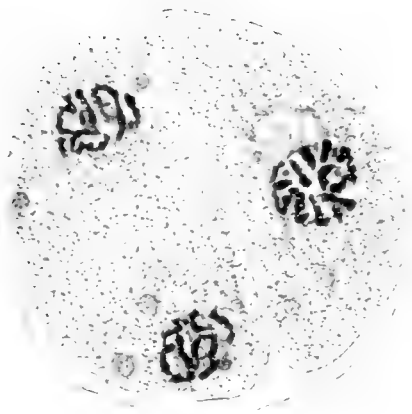

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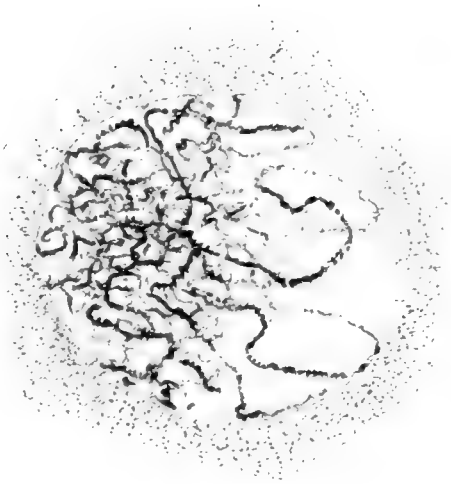

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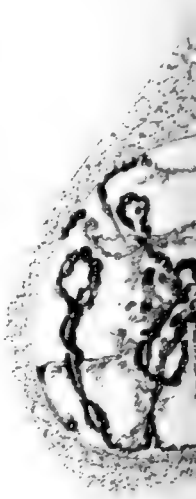

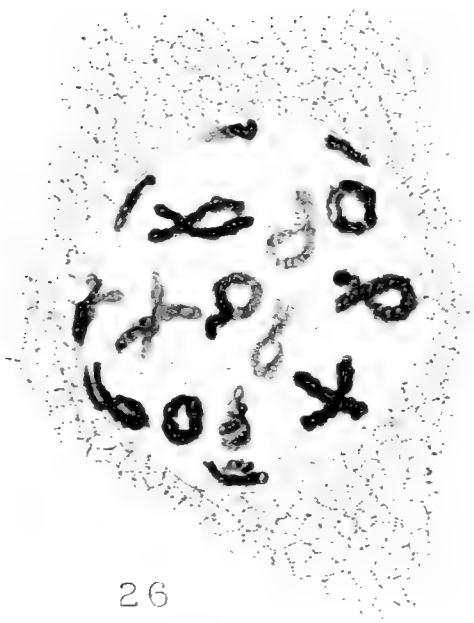

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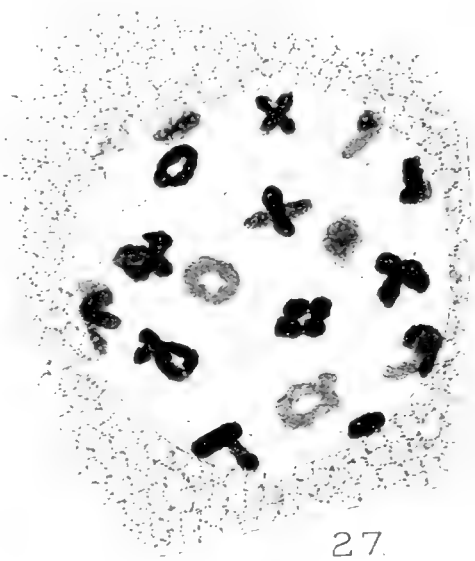

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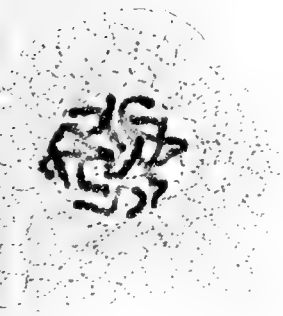

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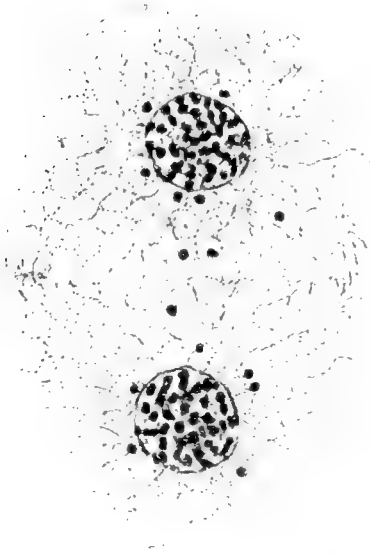

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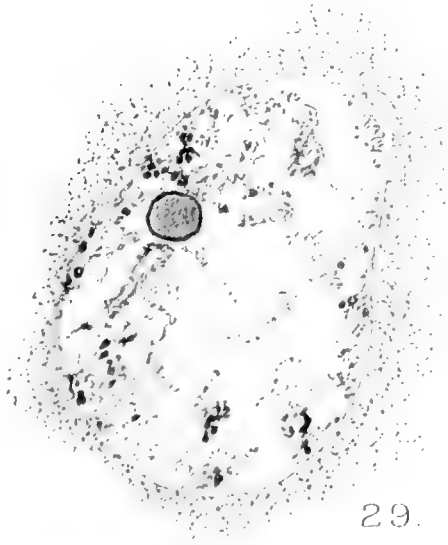

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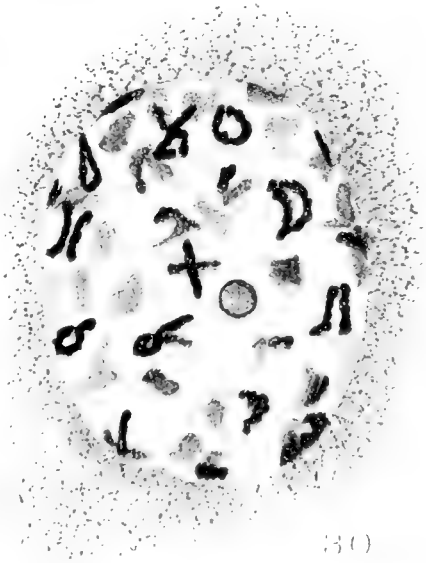

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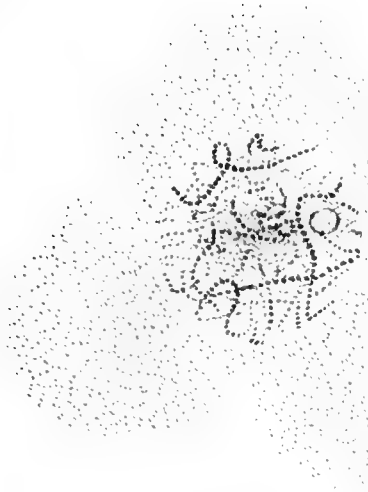

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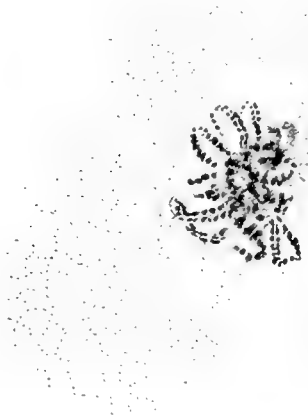

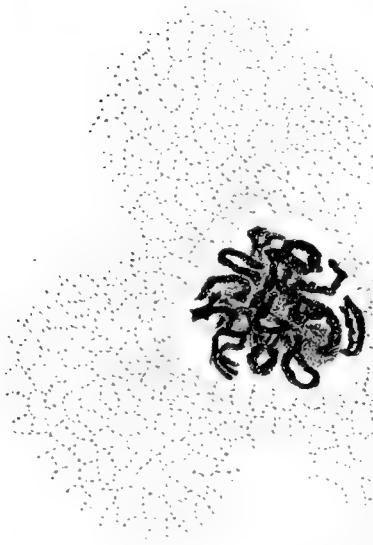

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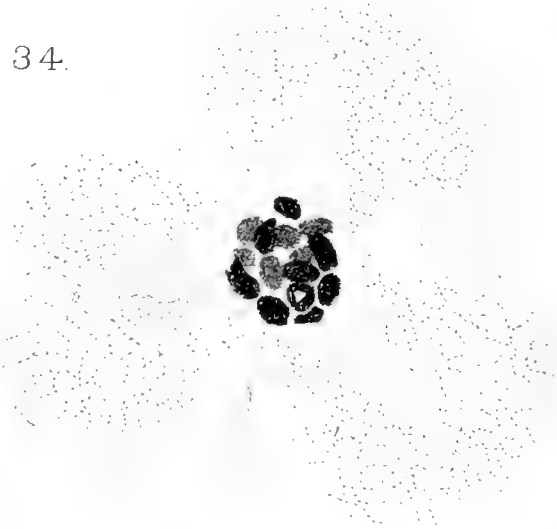

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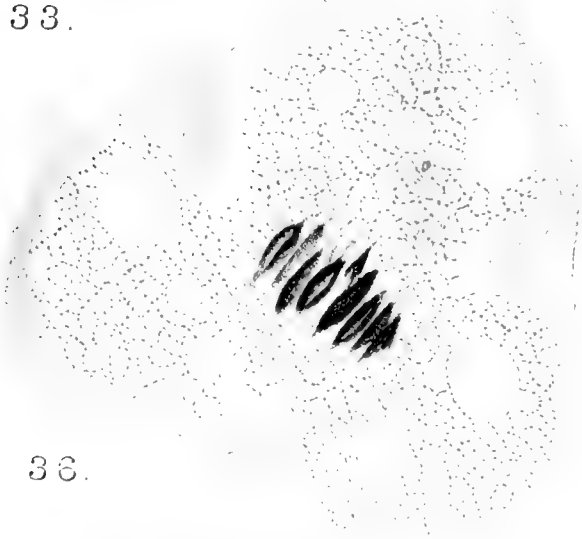

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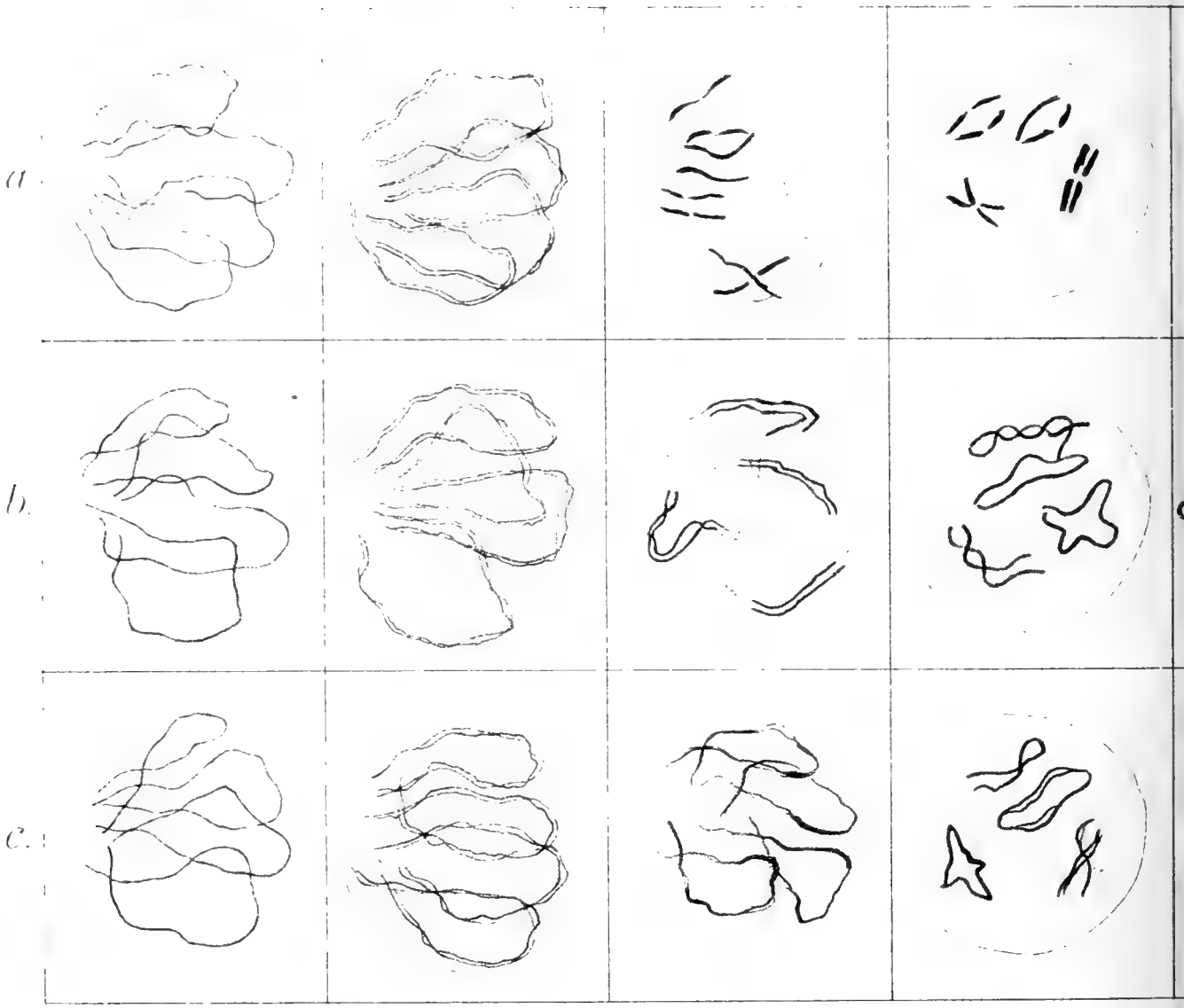

Diagrams illustrating three interpretations of th

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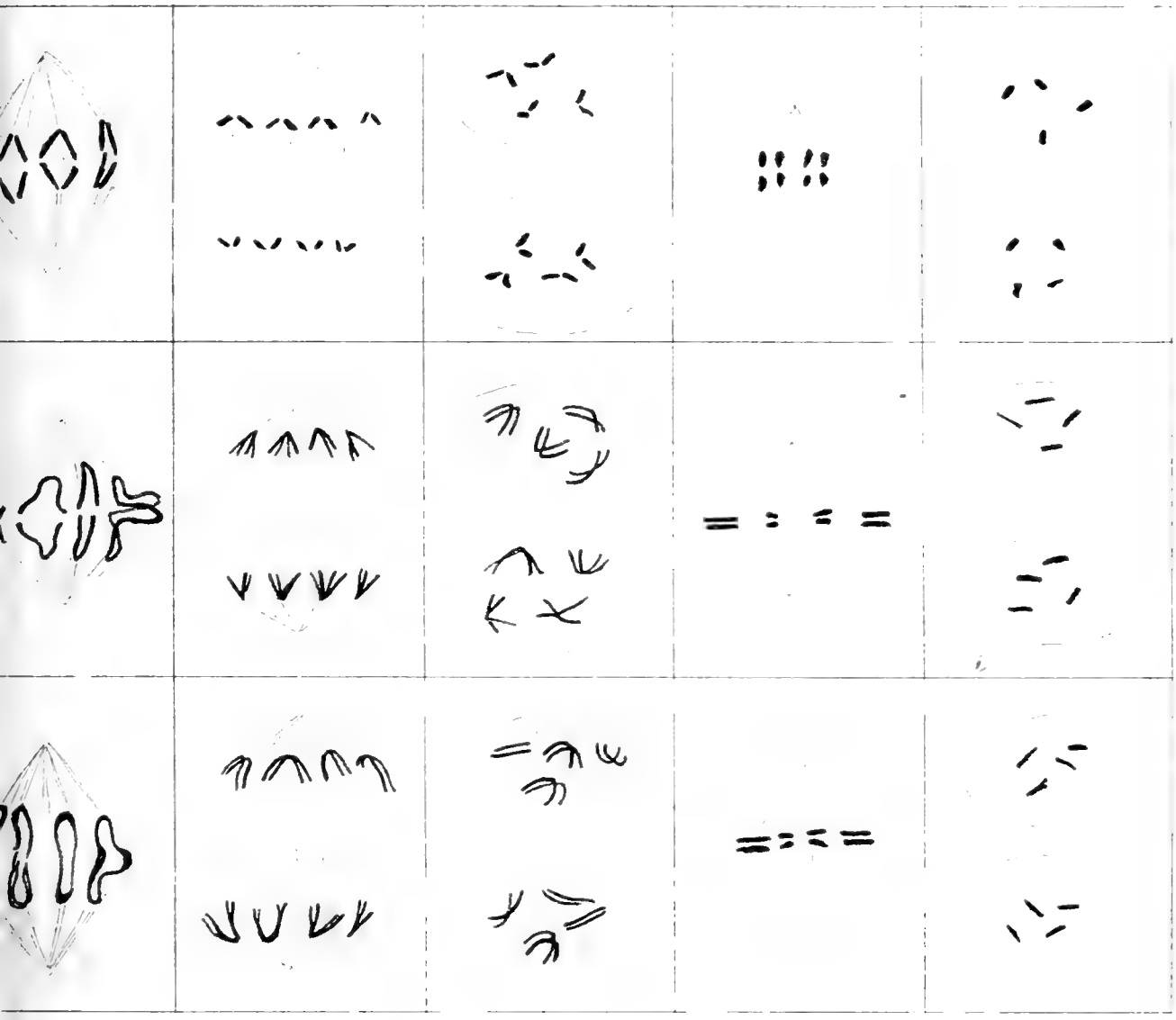

process of reduction in Animals and Plants.

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e views held by Flemming and others, and formerly by ourselves.

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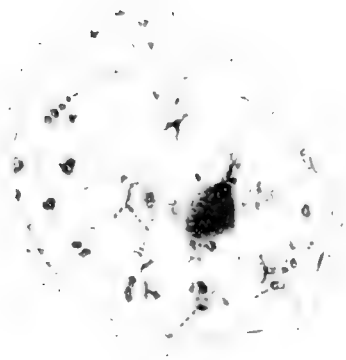

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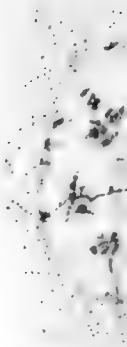

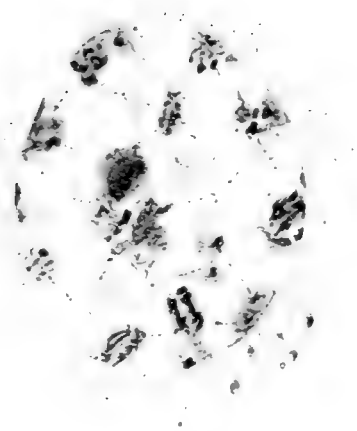

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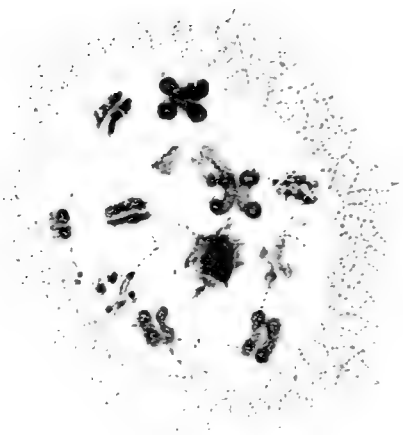

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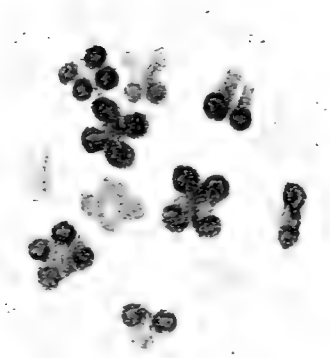

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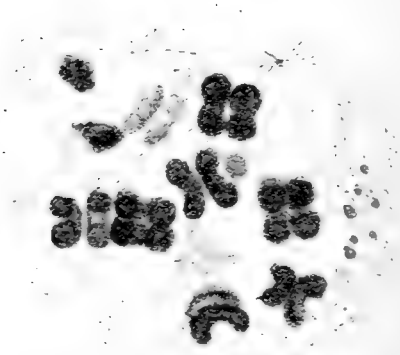

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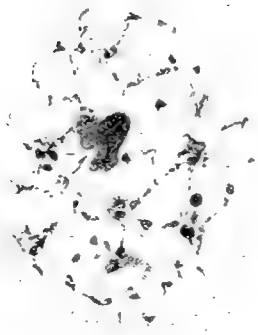

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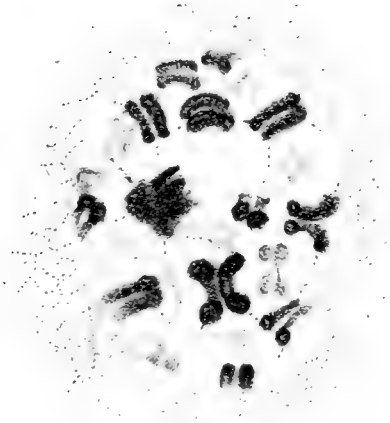

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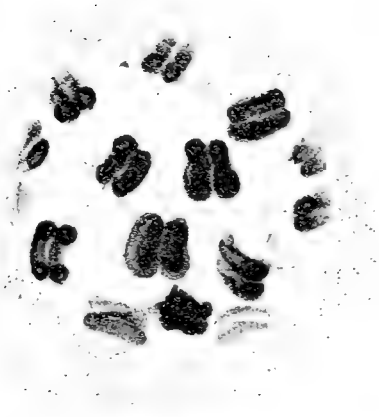

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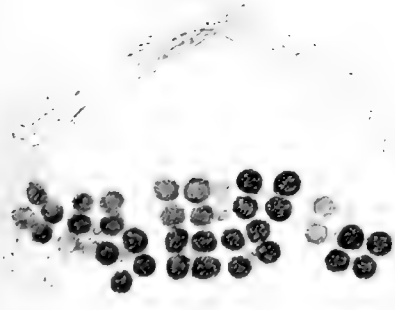

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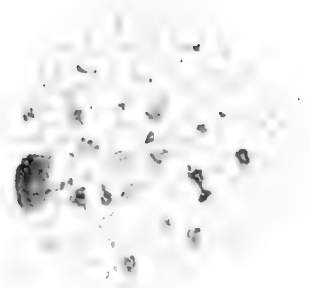

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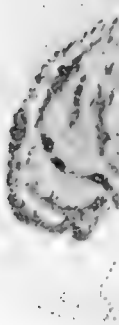

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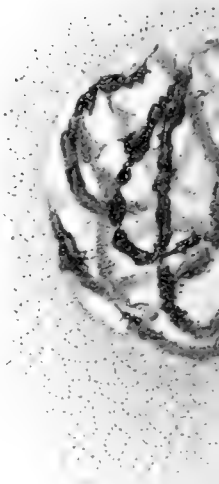

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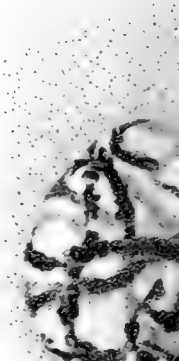

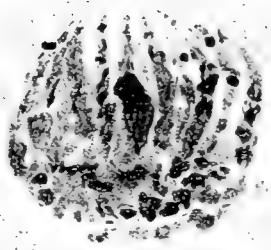

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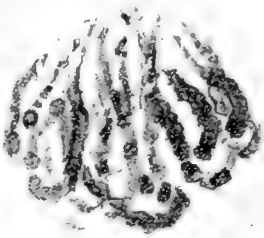

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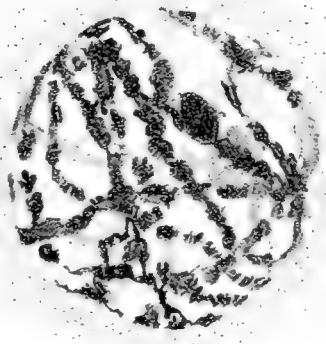

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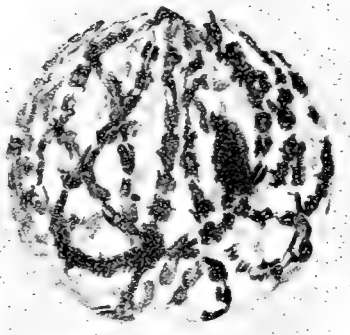

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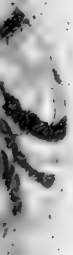

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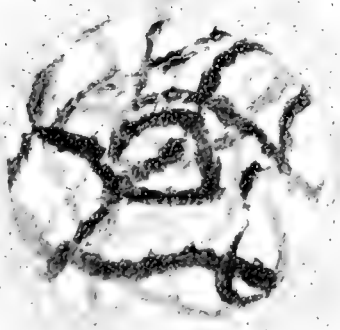

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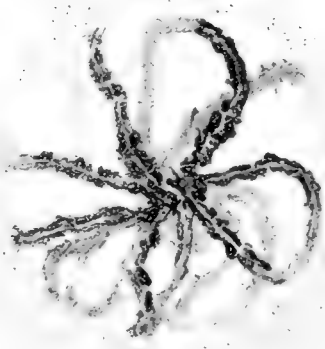

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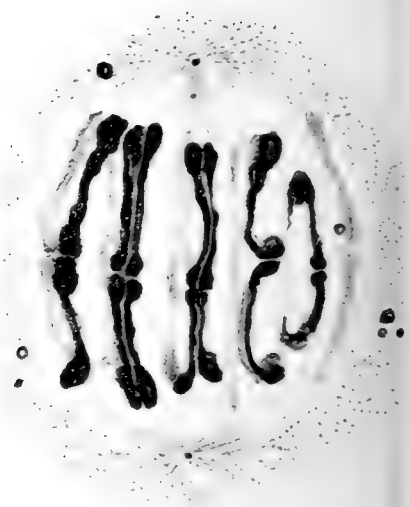

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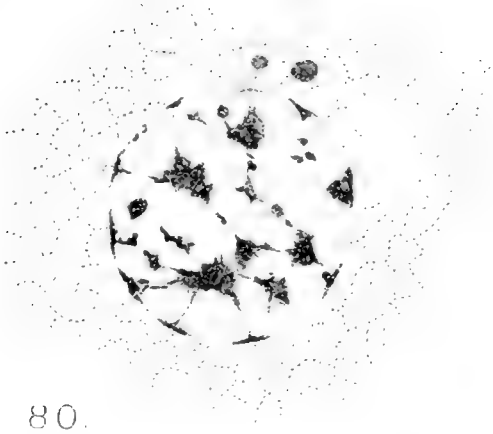

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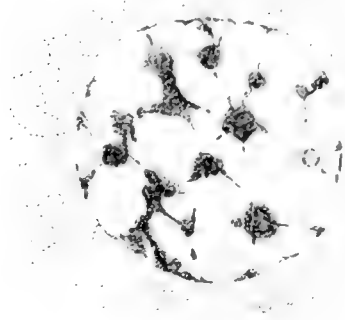

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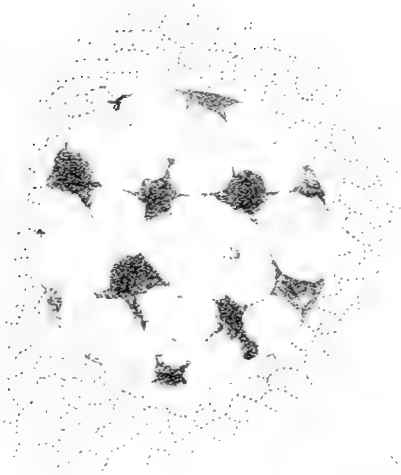

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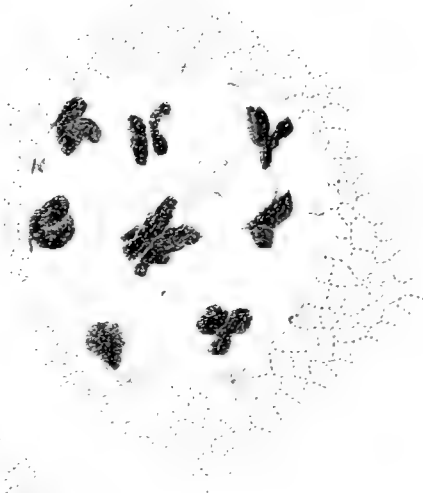

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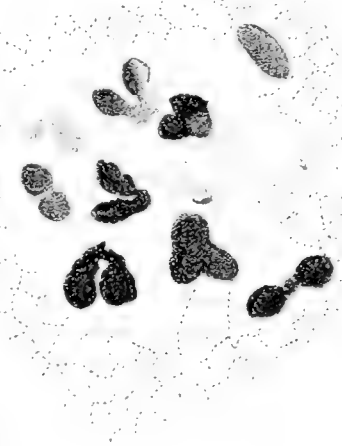

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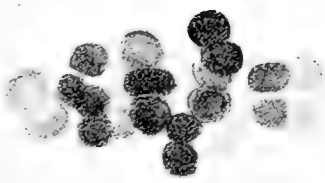

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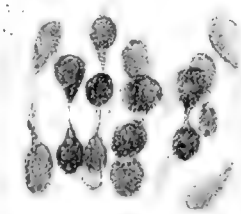

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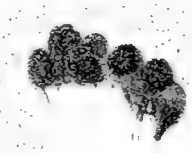

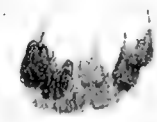

87.

On the Structure and Development of the Somatic and Heterotype Chromosomes of Tradescantia Virginica.

By
J. B. Farmer, F.R.S., and Dorothy Shove.

With Plates 42 and 43.

The object of this paper is to attempt to elucidate the series of changes that occur during the evolution of the chromosomes from the resting nucleus. We have expressly excluded all matter relating to the formation of the spindle, the cell wall, and other irrelevant structures from our account.

I. Somatic Mitoses.

Tradescantia virginica has proved a more suitable object for cytological study than might have been anticipated from the known difficulties it presents in the way of good fixation of the cell contents. This difficulty has been experienced by others as well as by ourselves. Thus Strasburger has commented upon the tendency of the nuclear contents to assume a condition during the prophase of the heterotype mitosis that obscures all the finer details of structure. We have found that Flemming's strong solution and Hermann's fixative give fair results when allowed to act for a long time, and the tissues are carefully washed and dehydrated afterwards. But on the whole the best material was yielded after fixing with a mixture of absolute alcohol and glacial acetic acid in the proportions of 2 : 1. The fixa-

tive was allowed to act for 15—20 minutes, and the tissues were then thoroughly washed with absolute alcohol, and then embedded as rapidly as possible by the usual methods.

We made a study of the cells of the root, in order to follow out the changes in the divisions of vegetative tissues. For this purpose plants were potted, and gently forced till they provided a plentiful crop of young roots.

While the nucleus is the resting state the chromatin is fairly evenly distributed throughout its substance, and there is a large nucleolus, often excentrically situated. The first signs of approaching mitosis is seen in a tendency on the part of the chromatin to aggregate into broad band-like areas, between which are left comparatively clear spaces. The nucleolus becomes replaced by a number of denser nucleoli which lie in close juxtaposition to the bands, though they are not arranged in any very definite order.

The band-like agglomerations of chromatin, when they first make their appearance, are not distributed throughout the nucleus, but are commonly visible in one region, whilst they fade away in another into the homogeneous granular arrangement characteristic of the resting nucleus.

It is important to notice that when a band is carefully examined the chromatin is seen to merely represent a closer and denser granular aggregation.[1] It is only at a much later stage that the spireme, with its single row of granules is formed. The contraction of the bands soon follows, and a reticulum, as shown in figs. 2 and 2*a*, is the result. The separate granules have now disappeared, and the chromatic reticulum appears merely to be rather irregular in thickness, and it readily takes the ordinary basic dyes. The next stage is marked by the more definite appearance of a spireme. This seems to be formed by the gradual breaking down of the original points of anastomosis, and the consequent restriction of the chromatin to a linear arrangement.

It is excessively difficult to ascertain whether a continuous

[1] Grégoire and Wygaerts ('Beihefte z. Bot. Centralbl.,' xiv) have observed similar arrangements in the roots of the plants examined by them.

filament is present. In many cases the evidence pointed strongly in favour of the existence of free ends, but it was not possible to make out any relation between them and the number of chromosomes to be ultimately produced. At this stage it was, however, clear that the arrangement of the chromatin within the linin filament (or filaments) was of that intermittent character to which the appearance of alternate stainable and non-stainable discs is due.

Closely following on this stage the granules or discs of chromatin can be sometimes made out to be double. We look on this as an indication of that longitudinal fission of the thread which only reaches its climax when the isolated chromosomes are arranged on the equatorial plane of the spindle—an event that happens long after the stage we are now describing. The fission is not very easily seen, but it can hardly be missed if looked for.

During the next phase the spireme undergoes a remarkable polarisation. Loops are formed in such a way as to make the "pole field" (Rabl) strikingly obvious (fig. 8). Within these loops the signs of longitudinal fission can sometimes be detected, though usually only with difficulty. The nucleoli at this stage are easily recognised as scattered through the region occupied by the polarised spireme thread, and we think the relation almost irresistably compels one to associate these bodies with the function of increasing the chromatin within the linin filament.

The filamentous structure now can with certainty be recognised as discontinuous (figs. 8—10), and a number of separate loops are readily distinguished. It is, however, not easy to determine whether their number is identical with that of the chromosomes, though this seems sometimes to be the case. The double arrangement of the chromatic beads in parallel series (indicating fission) was often observed at this stage (fig. 11).

The chromosomes now become capable of identification, and though, as we have said, we do not feel able to speak positively as to whether they have always been separated,

there can exist no doubt of the fact during this part of the prophase (figs. 12—14). Careful examination of the free ends will often indicate that each chromosome is really split, and this becomes very clear as the equatorial plate stage is reached (fig. 15).

The diaster is formed in the well-known fashion, by the separation of the longitudinal halves of the individual chromosomes and their distribution to the appropriate pole. During the anaphase irregularities are often encountered. Some chromosomes often seem to get away from the main groups, as shown in figs. 17 and 18.

When the number of the chromosomes of these nuclei is estimated one soon comes to realise that it is not constant. There can be no doubt whatever on this point, and, as it is of some interest, we may state we paid special attention to it, and made a very large series of drawings and countings of those examples that admitted of a reliable estimate being arrived at. The number varies from about twenty-six to thirty-three. The last was the highest number observed. As regards the lower numbers, we confined ourselves to those cases in which the razor had not touched the nuclei, in order to exclude the possibility of accidental removal of any of the chromosomes.

As the anaphase and telophase supervene, the chromosomes pass through the reverse series of changes already observed during the prophase. The vesiculation, long ago noticed by van Beneden, and since then confirmed by numerous observers, is strikingly shown in these Tradescantia nuclei. The chromosomes become thicker, and finally the chromatin is seen to be distributed as a cloud of fine granules through the linin band. At the same time the nucleoli are regenerated, and it is a significant fact that they always appear in the first instance in close connection with the chromatic bands, and they are much more numerous than during the later stages of telophase. This diminution in number is clearly effected by fusion or running together of the previously discrete nucleolar masses. The clear area that surrounds each

nucleolus in fixed preparations indicates a precipitation of the coagulable constituents which during life probably were of a fluid or viscous consistence. Hence, when the solidified matter is thrown down, the light aureole represents the fluid, non-precipitable remainder. Slowly the bands of linin, which contain the chromatin, continue to swell up till their apparent individuality is lost, as the equal spacing of the stainable substance necessarily obliterates the criteria of boundaries. But it does not follow that this obliteration extends to the real morphological, and still less to the physiological, limitations.

II. The Heterotype Mitosis.

The cells of the sporogenous tissue in the anther, just before they enter upon the two final (maiotic[1]) mitoses by which the pollen grains will be formed, are bound together into a compact tissue. The nuclei of the cells are large, but they do not exhibit that even distribution of chromatin which is often met with in other cells. The nucleus when carefully examined is seen to contain fibrils of chromatic linin. Sometimes (fig. 26) these are so arranged as to simulate more granular arrangement, but closer inspection shows the case to be otherwise. It is quite certain that during these early stages of prophase there is no continuous spireme present. The ends of the stainable threads can be clearly recognised. On the other hand, there is nothing recalling that differentiation into broad chromatic bands which forms so characteristic a feature of the ordinary somatic prophase. It is not clear that one is justified in laying too much stress on this difference. It may depend on accidental circumstances, such, for example, as the length of time that elapses between the telophase of one mitosis and the prophase of the next.

Soon the fibrillar structure becomes more dense, and the separate fibrils cannot with certainty be any longer identified.

[1] See Farmer and Moore "On the Maiotic Phase (Reduction Divisions) in Animals and Plants," 'Q. J. M. S.,' 1904.

If they are really present they must increase greatly in length, or else the coiling of the filaments now proceeding must be attended by end-to-end fusion (fig. 7).

During the prophase of this mitosis two "contraction" figures may be recognised; the first, appearing as the fibrillar arrangement, seems to give way to a more filamentous structure. Possibly the two circumstances may be in some way related, but at any rate after the contraction passes away the chromatin appears as a much coiled filament, while there is a clear alternation of stainable and non-stainable discs, as noted long ago by Strasburger and others. The stainable (chromatic) discs divide in such a manner as to bring about the fission of the thread, though the two halves do not, during prophase, divaricate much from each other.

A second point is easily established with respect to the filament after the first contraction is over; the coils, into which it is thrown, become very strongly polarised. Indeed, the effect is nearly as striking as in the case of animals (figs. 30—34). The loops thus formed and spread out can be easily examined, and they are clearly seen to be split longitudinally. This early longitudinal fission is of some importance, because it has often been regarded as diagnostic of the heterotype mitosis, but, as we have already seen it to be present during the earlier stages of the somatic division, it is obvious that this criterion, as a means of diagnosis, breaks down.

The polarisation of the spireme is also common to the heterotype and the somatic mitoses, but it does not seem possible to correlate the number of loops of the spireme with the final number of chromosomes to be produced. The polarised appearance, during which the spireme folds lie so regularly arranged within the nuclear wall, is, however, a transient phase. A second contraction of the thread follows it, and results in the balling together of the filament to one end of the nucleus, usually around the nucleolus. Whilst in this state (figs. 34 and 35) the longitudinal fission can still be seen, though it is becoming for the most part obliterated

owing to the re-fusion of the two halves into which the filament had commenced to split.

A considerable increase in thickness of the thread now occurs, and as the coils once more loosen the number of chromosomes that will be ultimately produced can be determined. The isolated lengths of the filament are partly bent, each limb showing a tendency to coil round the other, or two quite separate rods lie in close approximation. There is no doubt whatever that the paired structures thus lying in juxtaposition have been formed from different lengths of the spireme, and not by the shortening of the longitudinally-divided halves of single lengths. The cases in which they can be recognised as being formed from one loop, the sides of which have become closely adjacent to each other, coupled with the fact that the fission can still be recognised in each limb, sufficiently indicates the mode of origin of the parts of which the heterotype chromosome are made up, and shows that each is really a bivalent structure. But when the evidence of number is taken, it is less satisfactory than in most other cases. There is no doubt but that in this plant the number of the chromosomes is not constant during the heterotype division, and it certainly varies between twelve and sixteen; possibly the common failure of the plant to set seed may be related to this irregularity.

As the chromosomes advance towards maturity they separate from each other, and it is possible to observe other forms in addition to those just described that support the views here advocated as to their bivalent character. Fig. 39 shows a case, not very uncommon, in which the lowest chromosome is clearly not composed of parallel sides at all, but its components are adherent end-to-end, and showing this by the thin zone where they are attached together. This figure explains the presence of the long rod-like chromosomes that are sometimes seen on the spindle; such forms always ultimately divide across the middle zone (cf. fig. 44).

The majority of the chromosomes assume the form of oval closed rings, but they become so thickened as they congregate

at the equatorial plane of the spindle that their real form is not easy to discern. At this period all trace of the longitudinal fission is obliterated—at least we have never been able to recognise it with certainty, even in the best preparations.

The chromosomes next enter on the stage of the diaster. The ring-like ones sometimes break across the middle, leaving two half rings to travel to each pole. Often only one of two sides breaks at first, and then this frequently becomes almost straightened out, as though it were being forcibly pulled to the pole. There are many differences in the exact mode of division pursued, as might have been anticipated when dealing with viscous structures, but in principle the result is invariably the same. The chromosome, as representing a continuous length of the spireme, breaks transversely, and so different entire segments of the spireme are distributed between the two daughter nuclei.

Immediately after the separation of the daughter chromosomes from each other, they undergo a change which admits of the reappearance of the longitudinal fission. This was figured and described by Strasburger[1] some years ago, and we are quite in agreement with his statement of the facts (see our figs. 45—48). This peculiar occurrence has been several times observed in various animals, but its significance was not, until recently, properly appreciated. It is also of wider occurrence in plants than is often supposed.

A remarkable irregularity, similar to that described by Juel[2] for Hemerocallis, has been found by us[3] to occur in Tradescantia. This irregularity consists in the frequent omission of some of the chromosomes to reach the daughter nuclei with the rest of their fellows. Consequently they get left out in the cytoplasm when the two daughter nuclei

[1] Strasburger, "Reductions Theilung," etc., 'Histologische Beiträge,' vi, p. 51.

[2] Juel, 'Pringsheim's Jahrb. wiss. Bot.,' Bd. 92.

[3] Prof. Marcus Hartog has also observed the phenomenon in question, and kindly communicated his results to us.

become reconstituted. Sometimes they are found in the equatorial zone, but often they lie near the cell periphery or even in the cytoplasm. They do not appear, however, to give rise to small pollen grains, at least as a rule, but perhaps in most cases they degenerate.

In one example it was clear that the chromatic fragment thus left in the cytoplasm originated as a detached fragment of a chromosome.

III. Homotype Mitosis.

After the telophase of the heterotype division the nuclei do not revert to a resting condition. The chromosomes cannot, however, be identified as separate structures. They swell, and undergo those regressive changes that if completed would bring about the resting condition. Here and there signs of a double or parallel arrangement of chromatin granules suggests a persistence of the longitudinal fission. Then the mitotic activity is again resumed, the chromatic thread-work shortens and contracts, and the chromosomes themselves become easily recognised, although it is difficult and generally impossible to distinguish any signs of longitudinal fission at this stage. They are thinner and longer structures than those met with in the prophase of the former division, and they exhibit curious varicosities over their entire length. Finally, when they are arranged upon the spindle they adopt the same form of grouping as that characteristic of somatic cells. As in the latter the longitudinal fission now becomes unmistakeable, and the two halves are then separated and distributed to the two daughter nuclei.

It thus becomes evident that the essential phases in the heterotype mitosis whereby reduction by a sorting out of entire chromosomes is effected, are to be regarded as an intercalated series of events breaking the ordinary rhythmical sequences. The longitudinal fission begins during the prophase of the heterotype mitosis, but its natural outcome is postponed whilst the train of events runs off on a loop-line, the track

being once more rejoined after the true reduction has been effected. The normal process is again resumed at the spot where the divergence first occurred, and the longitudinal fission achieves its logical result in the equatorial plate of the homotype division.

EXPLANATION OF PLATES 42 & 43,

Illustrating Prof. J. B. Farmer's and Miss D. Shove's paper "On the Structure and Development of the Somatic and Heterotype Chromosomes of Tradescantia Virginica."

Somatic Divisions.

Fig. 1.—Resting nucleus, chromatin evenly distributed.

Fig. 2.—Nucleus showing definite strands of linin with chromatin granules.

Fig. 3.—Linin strands undergoing contraction.

Fig. 2A.—Nucleus showing reticulate structures.

Fig. 3A.—Breaking down of reticulum, with shortening and thickening of the strands.

Fig. 4.—Chromatin granules arranged in single rows in the linin strands, and strands in form of spireme.

Figs. 5, 6.—The linin strand further contracted.

Fig. 7.—A transition from above stages to the well-marked polarisation figure.

Figs. 8, 9.—Complete polarisation of loops of spireme strand, each loop representing a complete chrosome.

Fig. 10.—The loops have lost their polarisation, and are undergoing contraction. The nucleolus has lost the greater part of its stainable substance when this stage is reached.

Fig. 11.—Longitudinal fission clearly marked by the arrangement of chromatin in two parallel rows of granules.

Fig. 12.—Further contraction, which shows individual chromosomes lying at periphery of nucleus; longitudinal fission can be seen.

Fig. 13.—The chromosomes are scattered over the largest area they cover during their life history.

Fig. 13A.—Ditto.

Figs. 14, 15.—Formation of the equatorial plate.

Fig. 16.—Equatorial plate.

Figs. 17, 18.—Diasters.

Fig. 19.—The swelling up of the chromosomes at the poles, and formation of the cell plate.

Figs. 20, 21.—Later stage in swelling up of chromosomes, and the formation of nucleoli.

Fig. 22.—Two daughter nuclei in a resting condition.

Reduction Divisions (Maiotic Phase).

Fig. 23—Resting nucleus of pollen mother cell.

Figs. 24, 25.—Fibrillar arrangement of chromatin; early prophase.

Fig. 26.—Fibrillar arrangement evenly distributed.

Figs. 27—29.—First contraction figure.

Figs. 30—33.—The stages following on the contraction figure and effecting a polarisation of the spireme. Longitudinal fission to be seen.

Fig. 34.—Commencement of the second (synaptic) contraction.

Figs. 35, 36.—Further stages in the synapsis. The longitudinal fission clear in Fig. 35.

Fig. 37.—Loosening of the synaptic contraction.

Figs. 38—42.—Stages in the evolution of the chromosomes.

Figs. 43—45.—Illustrate the common types of chromosomes seen during the diaster.

Figs. 46—48.—The groups of daughter chromosomes showing the reopening of the longitudinal fission.

Figs. 49—53.—Various figures showing extra-nuclear chromosomes left behind at the reconstitution of the daughter nuclei.

Fig. 55.—Nucleus preparing for homotype mitosis.

Figs. 56—58.—Stages in the homotype mitosis. In Fig. 56 one pair of nuclei are seen in profile, and the other in polar view.

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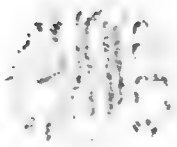

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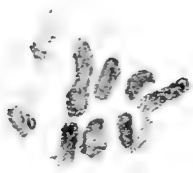

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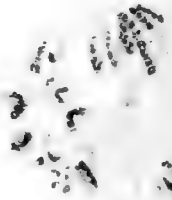

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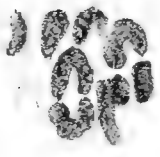

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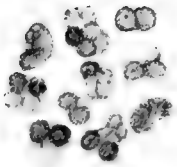

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On the Behaviour of the Nucleolus in the Spermatogenesis of Periplaneta Americana.

By

J. E. S. Moore, A.R.C.S., F.L.S.,

and

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With Plates 44 and 45.

The present communication has arisen as a by-product of the work upon the reduction processes in animals and plants, upon which the authors have for some time past been engaged.

Only a few cytologists have paid particular attention to the nucleolus, although this structure appears to be constantly present in both the male and female reproductive cells.

The great majority of writers on the subject of spermatogenesis figure the nucleolus in their drawings, but refrain from giving any definite account of its behaviour throughout the whole period of the maturation of the reproductive cells.

In recent years, however, the behaviour of this body has arrested the attention of a small band of cytologists on account of the part it may possibly play in some of the hitherto unexplained functions of the male reproductive element.

Henking appears to have been the first investigator to describe changes in the nucleolus of the reproductive cell in

his work on Pyrrhocoris ('Z. wiss. Zool.,' Bd. li, 1890), which led to its consideration as a modified chromosome, endowed with special functions, distinct from those of the normal chromosomes of the cell. Since Henking published this work, a few investigators have devoted their attention particularly to this subject, describing the nucleolus as an "accessory chromosome," and attempting to connect its function with such interesting problems as "determination of sex" and "heredity."

In the male reproductive cell of Periplaneta americana the nucleolus is conspicuous in almost all phases of the spermatogenesis, and consequently its actual changes can be studied without any great difficulty.

(I) The Premaiotic (Somatic) Divisions.—Throughout the somatic divisions, from the resting phase up to the complete differentiation of the chromosomes, the nucleolus is prominent as a large chromatic structure occupying a position inside the nuclear membrane. In the resting somatic cells the nuclei are large and oval in shape, and, on account of the small quantity of chromatin present, pale (fig. 1). The nucleolus (*nc.*) arises as an indefinite cloudy mass suspended in the linin reticulum. At first it stains very feebly, but rapidly becoming denser, finally retains the ordinary chromatin stains very powerfully (fig. 2). It always assumes a more or less spherical shape, and by the time that the nucleus exhibits the aggregation of chromatin granules in the angles of the linin reticulum (fig. 2), the nucleolus is by far the most conspicuous object in the cell.

In its early condition the nucleolus is usually irregular and ill-defined in outline, often somewhat stellate, the angular processes merging insensibly into the general linin reticulum.

Its structure at first is not homogeneous, there being frequently enclosed masses which stain more deeply than the general mass (fig. 1). As the chromatin granules increase in quantity the nucleolus becomes denser, staining more deeply, and finally reaching a stage in which it is as chromatic as a fully-formed chromosome. The outline of the nucleolus loses

its indefinite nature and becomes rounded off, and is readily distinguishable from the knot-like masses of chromatin usually termed karyosomes, but often described as a form of nucleoli. These bodies have nothing whatever to do with the nucleolus which is being described; they are collections of chromatic substance which are utilised, finally, in the formation of the chromosomes. The aggregations of chromatin rapidly assume the appearance of definite patches on the periphery of the nucleus (fig. 3), each patch being the early representative of a chromosome. Almost at the commencement of aggregation of the chromatin, it is seen that each patch is divided, this division being the line of separation of the halves of the chromosome on the spindle. When this stage is reached the linin reticulum begins to break down, and a considerable amount of linin substance collects in a mass round the nucleolus (figs. 3 and 4).

Delicate strands of linin continue to extend between the chromosomes, affording them support, until the appearance of the spindle. The chromosomes become denser and more sharply defined, and ultimately the extremities of each half are recurved, giving the divided chromosome the appearance of a tetrad (figs. 4—6).

As this condition is reached, owing to the size and density of the chromosomes, the nucleolus often becomes obscured, but its presence within the nucleus can be verified in a complete cell by counting the chromatic bodies within the nucleus. The number of chromosomes in the premaiotic division in P. americana is thirty-two, but at this stage thirty-three chromatic bodies may be counted within the nuclear membrane, one of these being the nucleolus.

The archoplasm (fig. 4, *a*.) at this time is differentiated as a dense cloudy mass of cytoplasm lying close to the nuclear membrane. Radiating striations soon appear in the archoplasmic mass, and the nuclear membrane, first becoming indefinite, finally disappears.

The radiating striæ now extend over the chromosomes which become massed together in the nuclear space, and the

nucleolus now undergoes fragmentation, the fragments being rapidly passed out towards the cytoplasm (fig. 5, *f. nc.'*).

The somatic spindle develops rapidly, and during the separation of the chromosomes on the equator of the spindle (fig. 6), the fragments of the nucleolus are seen to be undergoing rapid degeneration in the cytoplasm.

At the time of the appearance of a membrane between the two daughter cells, these fragments have, as a general rule, become indistinguishable.

The Second Maiotic (Heterotype) Divisions.—The nucleolus present in the nucleus of the spermatocyte is differentiated very soon after the immediately preceding somatic division, probably at the time of the reconstruction of the nucleus. It arises de novo, and not from the remains of the nucleolus present in the previous generation of cells.

The resting condition of the nucleus preceding the heterotype division differs markedly from the corresponding stage in the spermatogonium. This nucleus (fig. 7) is larger and usually spherical. The karyoplasm is more regular, consisting of a rather fine reticulum of linin, in which numerous small karyosomes (*k.'*) appear.

The nucleolus, a prominent, highly chromatic body (*nc.''*), lies in contact with the nuclear membrane, and usually exhibits a bifid condition, which gradually disappears.

A large mass of dense cytoplasm, the archoplasm (*a.*), becomes visible, lying close to the nucleus, and the appearance of this structure is the signal for the commencement of the remarkable series of changes about to take place in the arrangement of the nuclear contents, in connection with the phenemenon of reduction.

The whole nucleus becomes more chromatic, the increased deposition of chromatin granules rendering the linin reticulum sharp and distinct (fig. 8). Almost immediately the nuclear contents become polarised in the direction of an axis, passing through the archoplasm and the centre of the nucleus. The linin threads lying in the direction perpendicular to this axis rapidly break down, leaving a number of

meridional bands of linin which are densely infiltrated with chromatin granules (fig. 9).

This alteration in the disposition of the chromatin also affects the nucleolus. This body, being supported in the linin substance, becomes pulled out, at the time of the polarisation of the reticulum, into an elongated pear-shape (fig. 9). The elongated nucleolus at this stage often comes to lie in contact with one of the chromatic bands, and, as these bands shorten and thicken in the contraction of the nuclear contents, the nucleolus often closely simulates the latter in appearance (fig. 10). Shortly after the appearance of polarity, the nuclear contents contract away from the nuclear membrane, which becomes ill-defined, and it is then seen that the chromatin is arranged in a system of loops, sixteen in number, whose tapering, free extremities are gathered together at that portion of the periphery of the nucleus adjacent to the archoplasm (figs. 10, 11). This constitutes the first synaptic contraction of the heterotype prophase.

As the loops of chromatin contract the nucleolus also becomes shorter and thicker, the extremity remote from the archoplasm assuming the appearance of a dense blot on the surface of the nucleus (fig. 11). The loops of chromatin now begin to lengthen out so as to extend over the periphery of the nuclear space, and this takes place to such an extent that the appearance of polarity is lost. During this latter phase the long attenuated "tail" of the nucleolus is retracted, and the nucleolus assumes a spherical form, and apparently lies freely suspended in the nuclear sap among the skein-like mass of chromatin bands (fig. 12).

It remains quiescent in this condition throughout the following heterotype prophases, until the chromatic loops again contract towards the nuclear membrane at the point adjacent to the archoplasm (fig. 14). The nucleolus then undergoes fragmentation, giving rise to a number of small, highly-refractive, chromatic bodies, lying entangled in the bunch of contracted loops.

The heterotype spindle appears about this time as a

radiating striation, extending out from the archoplasm over the nuclear contents. The centrosomes rapidly move apart, and the chromatic loops fall asunder. The free ends of each of the loops are now seen to have fused together, giving rise to the typical heterotype ring chromosomes (fig. 15).

The fragments of the nucleolus, which have usually been imprisoned in the cluster of loops, are now liberated, and, as the spindle rapidly develops, they pass to the periphery of the nucleus, and are finally thrown out into the cytoplasm.

These fragments persist for some time, being visible after the homotype division has taken place as a number of small spherical masses of chromatic substance in the cytoplasm. They finally degenerate and undergo absorption (figs. 16—18).

The Second Maiotic (Homotype) Division.—Immediately after the completion of the heterotype division, the nuclei of the daughter cells do not enter a complete resting stage, the formation of the chromosomes proceeding almost immediately. The chromosomes appear as small angular masses, united by strands of linin, which are studded with granules of chromatin (fig. 19). The chromatin present in the linin strands gradually disappears as the chromosomes mature, being used up in this maturation process.

The chromosomes do not all develop at the same rate; it is usual to find perfectly-formed chromosomes in the same nucleus in company with the rudimentary angular masses (fig. 20). The homotype chromosomes like the somatic, exhibit a dual nature almost from the time of their differentiation, and, when mature, are very similar in appearance to those of the somatic nucleus. They consist of short, curved, thick rods, the swollen, free extremities of which give rise to an appearance of tetrads arranged on the periphery of the nucleus (figs. 20—22). The spindle appears at this time (fig. 22), and as the chromosomes pass on to the spindle they shorten up in such a manner as to completely lose their original tetrad appearance (fig. 23). They now appear on the equator of the spindle, in the divided condition, each as a

pair of rounded masses. As the two parts separate and move to opposite poles of the spindle, a strand of their substance remains as a connecting link for some time, giving them the appearance of a number of dumb-bells (fig. 24).

Throughout the homotype prophase no structure resembling a nucleolus has appeared in the nucleus, but the remains of the heterotype nucleolus still persist in the cytoplasm (figs. 19—25).

The reconstructed nuclei of the daughter cells, produced by the homotype division, present at first a dense, highly-chromatic appearance. An intermediate body (cf. Flemming) persists for some time, attached to which are the collapsed remnants of the spindle, and these finally separate and form an elliptical or rounded mass of dense cloudy cytoplasm, the nebenkern, a structure which probably takes part in the construction of the cephalic vesicle, and the tail of the spermatozoon (figs. 25—27).

The outer system of the homotype spindle-elements, as described by one of us in Elasmobranchs, is also often perceptible (fig. 26).

The Spermatid.—The nucleus of the daughter cell or spermatid rapidly loses its dense appearance (fig. 27), the chromatin gradually breaking down, and, as this proceeds, a well-defined, spherical, chromatic body becomes visible in the nucleus. It is smaller in size than either of the previously described nucleoli, in proportion to the reduced size of the nucleus in the spermatid.

The nuclear contents, at this stage, consist of a coarse reticulum of linin (fig. 28), suspended in which are the rapidly-disappearing, rounded masses of chromatin. At this stage the chromatic body or nucleolus of the spermatid (*n.'''*, figs. 28—31) is seen to lie in contact with the nuclear membrane, forming a very conspicuous object in the nucleus.

The coarse reticulum gradually breaks down into a finer structure, still supporting a few minute granules of chromatin. The nucleolus now undergoes fissure (fig. 31), one half remains

in contact with the nuclear membrane, the other passes inwards to the middle of the nucleus (fig. 32).

The cytoplasm, at this stage, contains a well-marked nebenkern, but the remnants of the heterotype nucleolus are by this time so altered as to be invisible.

The portion of the spermatid nucleolus which is still in contact with the nuclear membrane now passes through, and is extruded into the cytoplasm, where it appears as a rounded, highly chromatic mass (fig. 33), and is subsequently lost sight of in the liquor seminis.

The other portion of the nucleolus remains at the centre of the nucleus, and undergoes a slow process of degeneration, staining more and more feebly until it is finally lost sight of. The extra-nuclear portion of the nucleolus sometimes undergoes further fragmentation in the cytoplasm (fig. 34), but such fragments can be readily distinguished from the pale, degenerated fragments of the heterotype nucleolus, which may still be visible.

Before this stage is reached the centrosomes have not been recognised. They probably lie in contact with the nuclear membrane throughout the early phases of the metamorphosis of the spermatozoon, and, owing to the nature of the nucleus, this would explain the fact of their invisibility.

The formation of the cephalic vesicle, the axial filament, and the tail of the spermatozoon, and the behaviour of the nebenkern in connection with these processes are not yet sufficiently elucidated, and will possibly form the subject of a future communication.

The cytoplasm of the spermatid, which is not utilised in the formation of the spermatozoon, does not collect as a residual corpuscle in this insect, but undergoes a process of mucoid degeneration *in situ*. The extra-nuclear portion of the nucleolus is not affected by these degenerative changes of its surroundings, and large numbers of these chromatic bodies, derived from the different spermatids, may be seen among clusters of ripe spermatozoa in a ripe tubule of the testis floating in the liquor seminis.

The degeneration of the cytoplasm takes place before the spermatozoa can mature, and these pass through their final metamorphoses suspended in dense masses in the grumous liquid derived from the degenerated cytoplasm.

Conclusion.—As will have been seen from the preceding description, the behaviour of the nucleolus in the different stages of spermatogenesis of P. americana is distinctly interesting on account of the wide difference in its behaviour from that ascribed to similar structures by various authors in other animals. We find it, in fact, frequently discussed as an "accessory chromosome," differing from the ordinary chromosome both in structure and function.

In the somatic cell the nucleolus does not persist after the appearance of the spindle, but undergoes fragmentation, and is thrown out into the cytoplasm, where it undergoes degeneration. This process occurs in each successive somatic division, a nucleolus arising, de novo, in each of the daughter nuclei resulting from such division.

The operations described by Sutton as occurring in Brachystola ('Kan. Univ. Quart.,' vol. ix, No. 2, 1900), and by Miss Wallace, in Spiders ('Anat. Anz.,' Bd. xviii, Nos. 13 and 14), do not occur in the typical insect we have studied.

The nucleolus of the heterotype cell is not derived from that of the immediately preceding somatic cell, but arises anew in the earliest condition of the heterotype stage.

But in such cells essentially the same phenomena are repeated. The alteration in form of the nucleolus, in this case, appears to be due solely to the mechanical influences brought to bear upon an elastic structure enclosed in a nucleus, the contents of which are in a state of strain. Immediately this strain is relieved the nucleolus returns to its original spherical condition. With regard to the bifid condition of the nucleolus of the heterotype cell in its early stages, this is probably only an early manifestation of a tendency to division, such as occurs in the spermatid, analogous to the futile development of a flagellum in the

homotyle cell in Elasmobranchs as already described by one of us ('Quart. Journ. Micr. Sci.,' vol. xlvi).

As was the case in the somatic period, the nucleolus of the heterotype cell undergoes fragmentation prior to the division of the cell, and being extruded from the nucleus, undergoes degeneration in the cytoplasm.

This nucleolus is undoubtedly the homologue of the structure described by McClung as the "accessory chromosome" ('Zool. Bull.,' vol. ii, 1899); by Montgomery in "Pentatoma," ('Zool. Jahrb.,' Bd. xii); and by Paulmier, in "Anasa Tristis" ('Journ. Morph.,' supplement to vol. xv, 1899).

Toyama, in his investigations on Bombyx, and other Lepidoptera ('Bull. Coll. Agric., Imp. Univ. Japan,' vol. ii), describes the presence of two nucleoli in the heterotype nucleus, both of which are cast out into the cytoplasm to undergo degenerative changes. Platrus, in his work on Lepidoptera ('Internat. Monatschr. für Anat. med. Physiol.,' vol. iii), gives nothing very definite in his description of the nucleolus, but evidently noticed nothing approaching the behaviour of a chromosome in these structures.

In his researches upon Caloptinin femur rubrum and Cicada tibicen ('Bull. Imus. Comp. Zool., Harvard Univ.,' vols. xxvii, xxix, 1895, 1896), Wilson describes the reaction to stains of the different nuclear elements. He found that under certain conditions the nucleoli stain differently to the chromosomes. He also noticed that in Cicada the nucleoli in the heretotype all underwent fission, and were finally extruded. In the present case material was used which had been fixed by various methods,—by Flemming's fluid, Hemann's fluid, corrosive acetic, Rabb's method, and van Rath. It was stained either by Flemming's triple method or Heidenhain's iron hæmatoxylin, counterstained with Orange G, but in all cases the nucleolus was stained in the same manner as the chromatin.

The nucleolus of the spermatid appears to be differentiated directly from the chromatin of the reconstructed daughter nucleus immediately after the homotype division, and the most feasible explanation of the process which follows is, that it is

carried out in order to get rid of a portion of the chromatin in the spermatid.

The extra-nuclear chromatic body has often been figured, but less frequently described. Hermann describes a chromatic structure in the spermatid, which is probably the homologue of the one under consideration ('Arch. f. Micr. Anat., Bd. xxxiv). One of us, during investigations on mammalian spermatogenesis, have described the nuclear origin of the extra-nuclear body in the spermatid of the rat ('Internat. Monatschr. f. Anat. u. Physiol,' Bd. xi, 1894). But until more is known of the behaviour of the nucleolus in the late phases of both spermatogenesis and oogenesis, in different types, all attempts to draw theoretical conclusions on the function of this body, and the part it plays in the problems referred to in the introductory portion of this communication, must be of little value.

EXPLANATION OF PLATES 44 & 45,

Illustrating Mr. J. E. S. Moore's and Mr. L. E. Robinson's paper "On the Behaviour of the Nucleolus in the Spermatogenesis of Periplaneta americana."

All the figures were drawn under Zeiss' 2 mm. Apochromatic Immersion, 1·40 n. a., with No. 18 ocular.

nc.' = nucleolus of somatic division. *nc.''* = nucleolus of heterotype division. *a.* = archoplasm. *k.* = karyosomes. *f. nc.'*, *f. nc.''* = fragments of the nucleolus in the somatic and hetereotype divisions respectively. *nc.'''* = nucleolus of spermatid. *in. nc.'''* = intra-nuclear portion of spermatid nucleolus. *ex. nc.'''* = extra-nuclear portion of spermatid nucleolus. *B. in.* = intermediate body of Flemming. *N.* = Nebenkern.

PLATE 44.

Fig. 1.—Somatic cell in resting condition, showing nucleolus in early state.

Figs. 2—4.—Somatic cells showing differentiation of chromosomes, with condition of the nucleolus during the somatic prophase.

Fig. 5.—Somatic cell showing development of the spindle and extrusion of the fragments of the nucleolus.

FIG. 6.—Somatic cell with fully formed spindle. Fragments of the nucleolus in the cytoplasm.

FIGS. 7, 8.—Heterotype cells in early prophase. Nucleolus bifid.

FIG. 9.—Heterotype cell in which polarization has commenced. The nucleus has undergone extrusion.

FIG. 10.—Heterotype cell in condition of synapsis. Loops contracting in direction of archoplasm.

FIG. 11.—Heterotype cell in late synaptic phase. Nucleolus reassuming spherical condition.

FIG. 12.—Heterotype cell in which the loops of chromatin are extruded out on the periphery of the nucleus, the nucleolus lying free among the loops.

FIG. 13.—Heterotype cell. Later stage than Fig. 12.

FIG. 14.—Heterotype cell. The chromatic loops are contracting to form the heterotype ring chromosomes.

FIG. 15.—Heterotype cell. Ring chromosomes separating. Spindle developing. Nucleolar fragments passing out into the cytoplasm.

FIG. 16.—Heterotype cell. Chromosomes dividing on the spindle. Nucleolar fragments in the cytoplasm.

FIG. 17.—Heterotype cell in stage of diastes.

FIG. 18.—Heterotype cell in stage of late diastes. A cell membrane has appeared, dividing the cell into two daughter cells.

PLATE 45.

FIGS. 19—21.—Homotype cells showing stages of differentiation of the chromosomes. Fragments of heterotype nucleolus in the cytoplasm.

FIG. 22.—Homotype cell. Chromosomes going on to the spindle.

FIG. 23.—Homotype cell. Spindle with chromosomes on equator.

FIG. 24.—Homotype cell. Stage of diaster with dumb-bell-shaped chromosomes.

FIG. 25.—Homotype cell. Late diaster. Daughter cells separated, showing Flemming's intermediate body. Fragments of heterotype nucleolus in the cytoplasm.

FIG. 26.—Homotype cell. Later stage than Fig. 25, showing reconstruction of the nuclei in the daughter cells.

FIG. 27.—Spermatid showing nebenkern. Chromatin breaking down.

FIG. 28.—Spermatid. The nucleolus is differentiated. Some fragments of heterotype nucleus still visible in the cytoplasm.

FIG. 29.—Spermatid. Later stage than Fig. 28.

FIG. 30.—Spermatid. Nucleolus lying in contact with nuclear membrane prior to fission.

FIG. 31.—Spermatid. Nucleolus divided.

FIG. 32.—Spermatid. Nucleolus divided. Halves separating.

FIG. 33.—Spermatid. Nucleolus divided. The extra-nuclear portion has just been extruded; intra-nuclear portion degenerating within the nucleus.

FIG. 34.—Spermatid. Nucleolus divided. The extra-nuclear portion has undergone fragmentation in the cytoplasm. The intra-nuclear portion has become almost invisible.

FIG. 35.—Spermatid. Extra-nuclear chromatic body well defined. The intra-nuclear portion has vanished.

FIG. 36.—Spermatid. Showing extra-nuclear chromatic body in two fragments.

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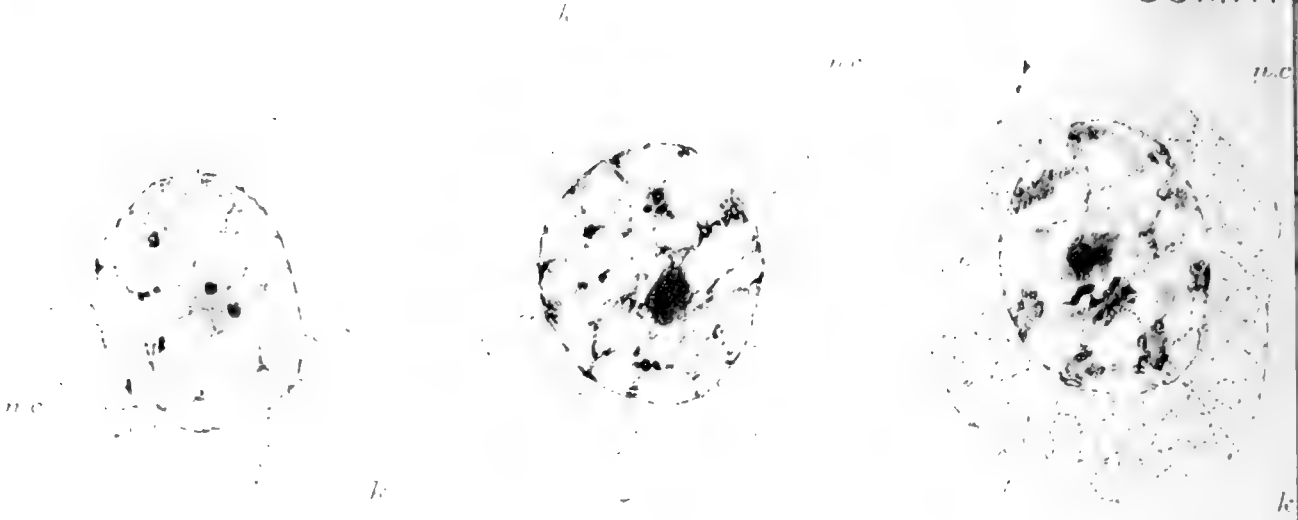

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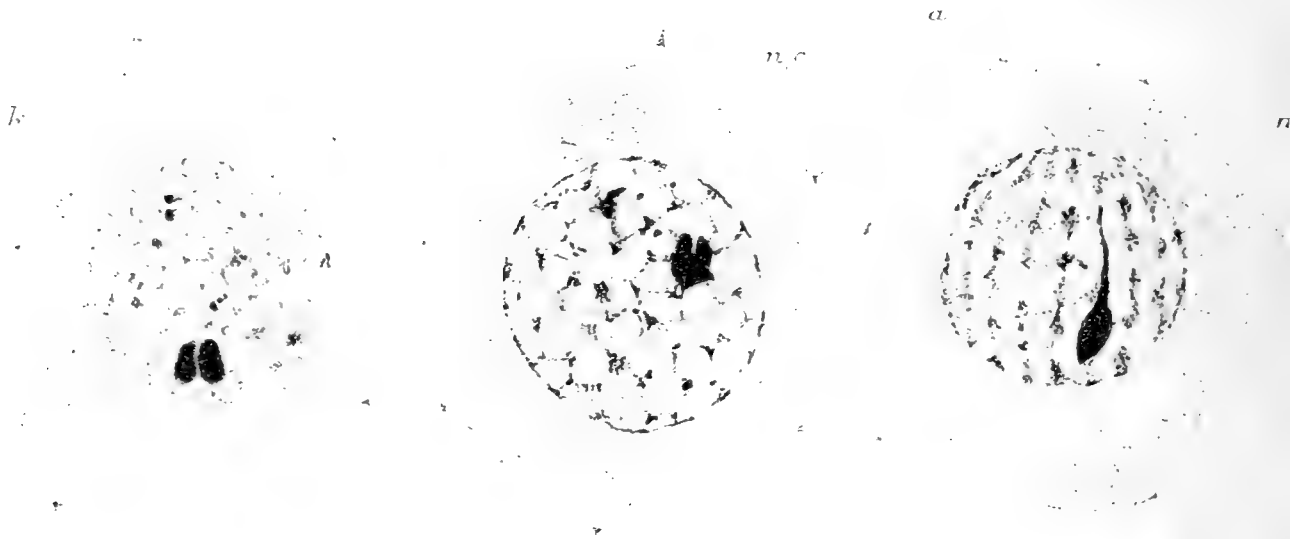

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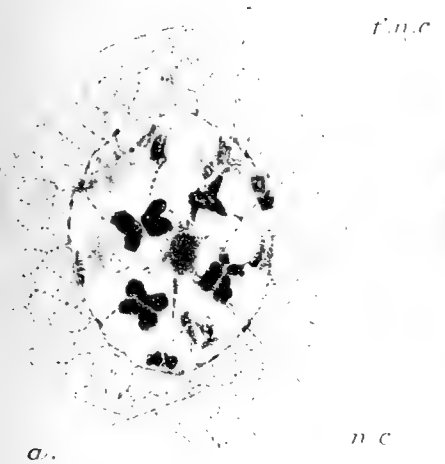

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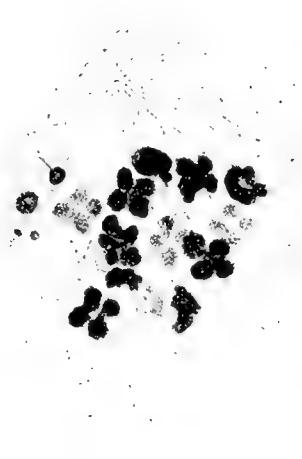

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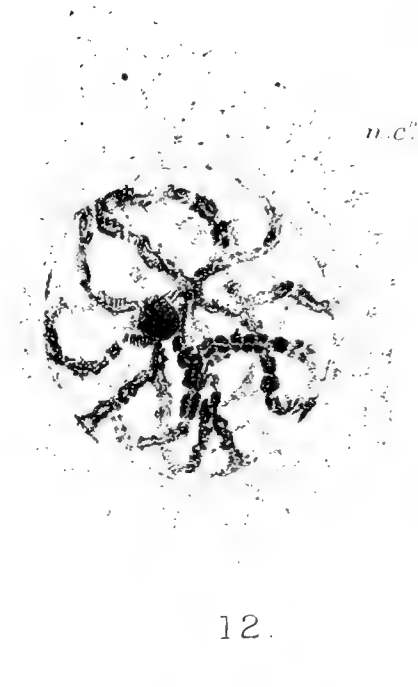

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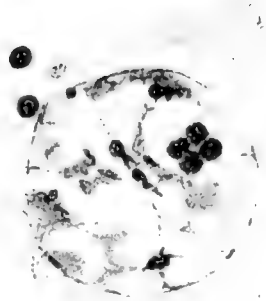

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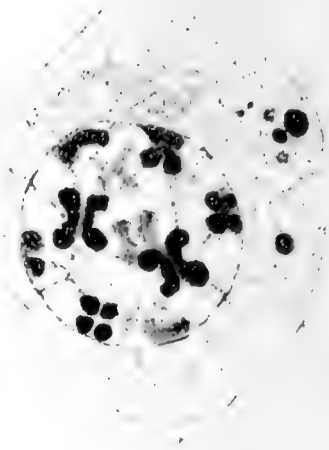

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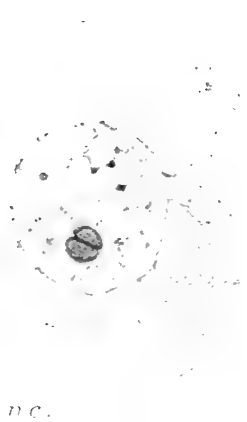

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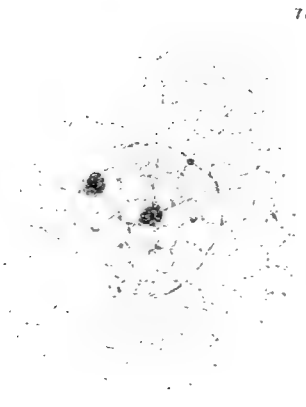

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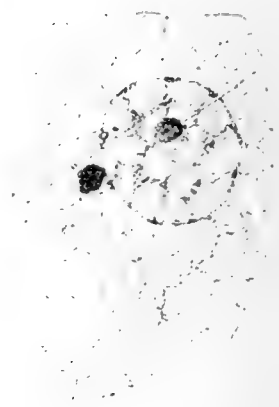

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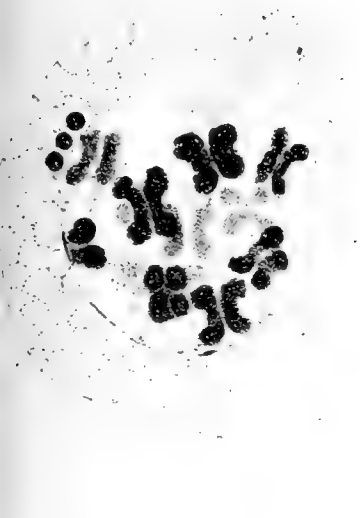

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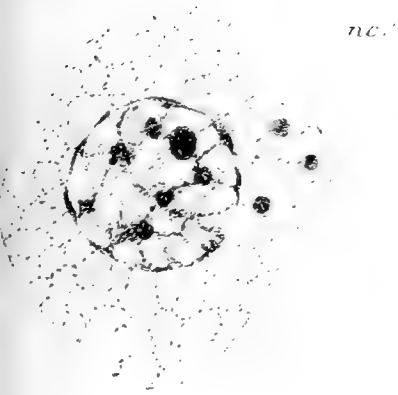

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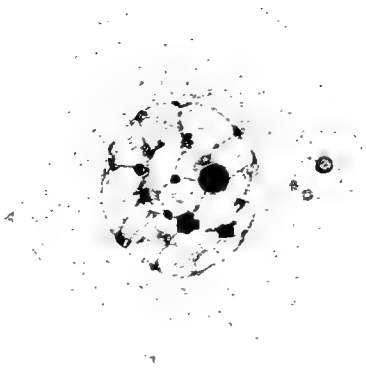

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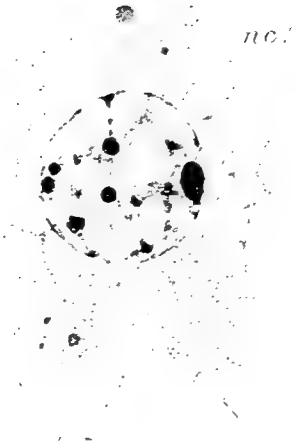

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On Some Movements and Reactions of Hydra.

By

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This study of the movements and reactions of Hydra was undertaken at the suggestion of Professor H. S. Jennings, whose own researches ('97—'02) on the reactions of protozoans have added new interest to this line of work.

Trembley (1744) described the normal movements of Hydra in considerable detail. Further observations on the same subject were made by Baker (1743), Rösel von Rosenhof (1755), Marshall ('82), and Zoja ('90). The works of Baker and von Rosenhof have not been accessible to me. Wilson ('91) made a detailed study of phototaxis in Hydra, while Pearl ('01) investigated its behaviour toward the constant electric current.

Normal Movements.—Trembley (l. c.) made a thorough study of the movements of Hydra, and his description of them is fairly complete. Hydra is usually attached by its foot to some solid substratum, as a submerged stem or branch, or a floating leaf, less frequently to the surface film. When not so attached it is helpless, for it has not the power of swimming. When attached the body is usually moderately expanded, seldom extremely so. In Hydra viridis the tentacles, also moderately expanded, extend obliquely outward and forward, forming the framework of a sort of funnel with the hypostome at the bottom. In Hydra fusca, and especially Hydra

grisea, where the tentacles have enormous capacity for expansion, they sometimes hang down in great garlands into the water, the individual tentacles often so thin that even under a lens they are barely visible.

Even when undisturbed Hydra contracts at intervals. This contraction is very sudden and rapid, while the expansion, which almost immediately follows, is gradual and slow. The contraction may involve both body and tentacles. It may, on the other hand, be restricted to either body or tentacles, or even to a single tentacle. The contraction occurs at much

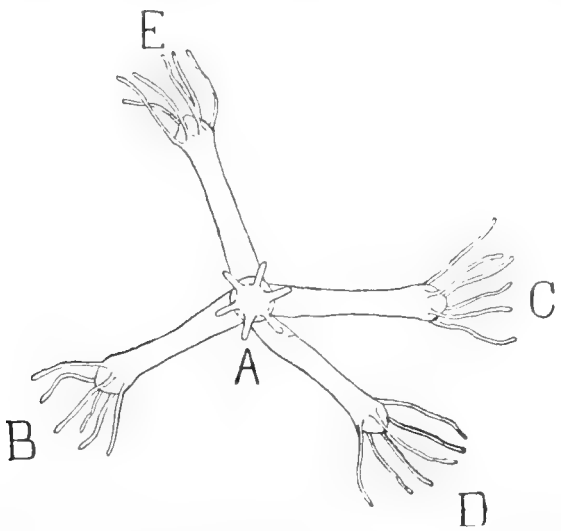

Fig. 1*a*.—Positions occupied by Hydra after successive contractions. For explanation see adjacent text.

more frequent intervals in Hydra viridis than in the other forms; Hydra viridis is also in most other respects the most active form.

On closely observing Hydra viridis for a period of from three to four hours it was observed that many of these contractions were really not spontaneous, but due to slight tremors produced by occurrences very easily overlooked. Such were the slamming of a door in some remote part of the laboratory, some person walking across the room on the floor above, and so on. Nevertheless, there remain many contractions which are evidently, as Marshall (l. c.) suggests, the results of internal physiological changes. These may very properly be termed spontaneous.

After each contraction Hydra soon expands again, but toward a different direction from what it previously occupied —a fact to which my attention was called by Professor Jennings. Let us suppose that the Hydra was previously standing with its long axis oblique to the substratum. If now, for any reason, it contracts, it soon begins to expand again, at first perpendicularly to the substratum, then the body flexes more or less in a new direction, so that when expansion is completed the head and tentacles are directed into a region different from that which they occupied before. As illustrated in Fig. 1*a*, when A represents the contracted condition the Hydra may occupy, successively, positions B, A, C, A, D, A, E, etc.

It may be useful to give here a case from actual observation.

A Hydra viridis was placed in a small dish under a dissecting microscope and left undisturbed for half an hour. Then its "spontaneous" movements were recorded. Precautions were taken to prevent disturbance of the dish in any manner. For the sake of brevity in writing the record the plane of the microscope table was looked upon as a map. Thus "north" means the side away from the observer, "east" lies to his right, etc. A movement "upwards" means a movement toward the surface of the water.

Here is a portion of a record made in this manner.

A.M.

9.23. Hydra contracted. Expansion to south by west, then west.

9.26. Contraction. Slight expansion to west. Contraction.

9.27. Expansion to north. Swaying to west.

9.28. Swaying to north-west.

9.31. Contraction. Expansion to south-east.

9.35. Partial contraction. Rest. Total contraction. Expansion to west, slightly north.

9.36. Contraction. Expansion to east, slightly south, and strongly upward.

9.38. Contraction. Expansion to north-east.

And so forth for three hours.

The extended Hydra may also change the direction of its long axis without a general contraction, by mere flexion of the expanded body. Sometimes the change from one oblique position to another is brought about by first swaying to the vertical, and then to the new oblique position. Quite as often, however, it occurs through circumnutation around the attached foot. In this case there appears first a contraction of the ectoderm on one side near the foot. This contraction then travels towards the hypostome in slightly spiral form. The Hydra, in this manner, slowly swings around, the body curved into a complete loop or even beyond (Fig. 1).

It can be seen that by either of these methods Hydra extends its body successively in many different directions in

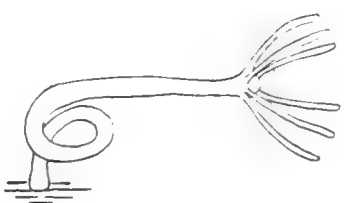

FIG. 1.—Hydra viridis changing position of body by a spiral contraction near the foot.

a comparatively short space of time. It is thus enabled to explore a relatively large space, and so greatly increase the probability of its chances of capturing prey. Herein lies, undoubtedly, the biological significance of the behaviour described.

These intermittent spontaneous contractions and expansions are much more frequent in Hydra viridis than in the other species. Marshall (l. c.) is probably correct when he correlates this fact with the shorter tentacles of Hydra viridis. The other species have long thread-like tentacles often extended to a length several times that of the body. By swaying these to and fro they can explore a large territory without any movement of the body, while in a quiet Hydra viridis the tentacles have very little spread.

When one observes Hydra (again especially Hydra viridis) for a longer time one is impressed with the fact that it can and does move from place to place with considerable rapidity. If a quantity of freshly collected Ceratophyllum is put into an aquarium that is unequally illuminated, the Hydra present will wander from the Ceratophyllum to the better lighted wall of the aquarium often within twenty-four hours. As Hydra cannot swim this involves a rather circuitous journey. But even without such a directive stimulus its movements are considerable. For example, a green Hydra was placed in a glass dish, and this was set over a sheet of ruled paper on the laboratory table. The lines of the paper were so numbered that the position of the Hydra could, at any time, be charted on a second piece of ruled paper similarly numbered. The chart of the journeys of this Hydra is shown in Fig. 2. Although the illumination was not entirely equal from all sides yet it was not one-sided enough to influence the movements. At all events the record shows no such effect.

Now, how are these movements brought about? The method can easily be seen by placing a single Hydra in a small dish and observing it under a dissecting microscope. The body, expanded and with expanded tentacles, bends over to one side. As soon as the tentacles touch the bottom they attach themselves and contract. (Zykoff ['98] claims that this attachment of the tentacles is by means of pseudopodia, but during observations covering many months I have never seen the formations he figures.) Now one of two things happens: (1) The foot may loosen its hold on the bottom, and the body contract. In this manner the animal comes to stand on its tentacles with the foot pointing upward. The body now bends over again until the foot attaches itself close to the attached tentacles. These loosen in their turn, and so the Hydra is again in its normal position. The successive steps of the movement are illustrated in Fig. 3. Trembley described the movement, and illustrated it (l. c. Memoire 1, Pl. 3, figs. 1–9); (2) In the other case the foot is not detached, but glides along the bottom until it stands close to the tentacles, which

now loosen their hold. The result in either case is the same. By one such manœuvre Hydra sometimes travels a distance several times its own length when contracted. Hydra is further able to make slower journeys by gliding about on its foot without aid from the tentacles. This movement is very

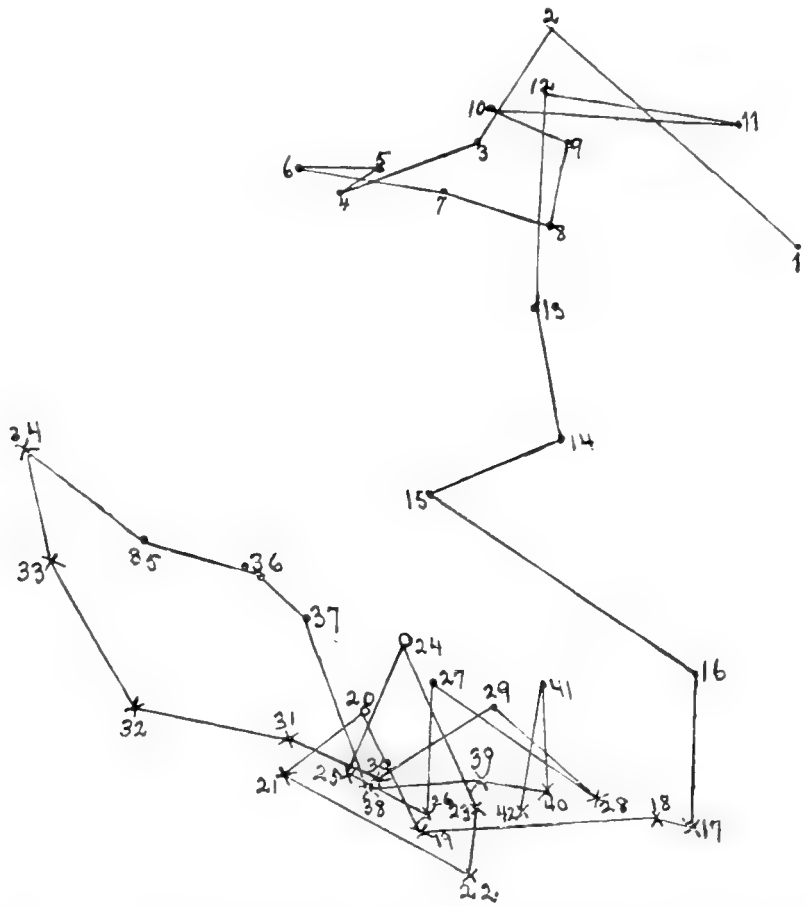

FIG. 2.—Diagram showing movement of a Hydra viridis in absence of any directive stimulus. The figures represent positions of the Hydra when observations were made. For further explanation see text.

slow, and noticeable only on very close observation. Nevertheless, Hydra travels considerable distances by means of it.

It has been stated above that Hydra is often found hanging from the surface film. A slight touch to such a Hydra will usually loosen its hold and cause it to fall to the bottom, but this is not always the case. Trembley (l. c. Mem. 1, p. 77, et

seq.) studied this phenomenon very carefully, and pointed out that during such suspension the basal disc of Hydra rests at the base of a capillary depression of the surface film. The disc, however, is above the surface film and dry. He compares the suspension with that of a pin or similar object when carefully let down into a vessel of water. These observations can easily be verified. Scourfield ('01), apparently without knowledge of Trembley's work, investigated the same subject, and came to practically the same conclusions as Trembley. He observed further, however, that there was present on the disc, in such cases, a gelatinous substance, often extending beyond the disc in minute strands. He believes that this substance, as water-repellent, is essential to the process of suspension. The fact that, in some cases, he found it difficult to force Hydra to leave the surface film adds weight to the view. I have myself seen a number of such cases. But Trembley's experiment with the pin shows that even without such a repellent substance the suspension is easily explained.

I have observed in a number of other cases a different method of suspension. In these a large air-bubble was attached to the basal disc, and this bubble apparently kept the Hydra afloat. It was, moreover, far from easy to remove this bubble or to break it. It seems to be surrounded by a tough substance, very probably of the same nature as that observed by Scourfield. Suspension from a longer thread, such as Scourfield reports, I have never seen.

Reactions to Mechanical Stimuli.

As previously mentioned Hydra viridis contracts and expands at much more frequent intervals than the other species observed. All its other movements are also more prompt and decisive. Hydra grisea and Hydra fusca are very sluggish both in responding to stimuli and in recovering after their removal. Furthermore, their tentacles expand so greatly that they form tangled masses that interfere seriously with the accuracy of results. I have therefore used Hydra

viridis almost exclusively in these experiments, and the descriptions apply entirely to that species. I did, however, experiment enough with the other forms to find that their reactions are practically the same.

Variation in Sensitivity.—A great variation in sensitivity to stimulation between different individuals became immediately apparent in the work on the reactions of Hydra. Many specimens proved useless for such work, because even the slight disturbance on the surface of the water caused by the breathing of the operator as he bent over the microscope produced immediate and complete contraction. On the other hand, there were specimens in which an actual wound had to be produced in the ectoderm before they responded at all. Between these two extremes all degrees of sensitivity occurred. The terms "weak" and "strong," as applied in this paper to stimuli, have, therefore, only a relative meaning. What is "weak" to one specimen may be exceedingly "strong" to another.

Non-localised Mechanical stimuli.—If the watch-glass containing a Hydra is slightly jarred the Hydra contracts. The same result occurs after any disturbance of the surface of the water. If after one such stimulus the dish remains undisturbed the Hydra soon expands again.

Now what is the result if the Hydra is subjected to rhythmically repeated, uniform mechanical stimuli? Such a succession of stimuli is best applied by tapping the stage of the microscope with some metal body at intervals of about one second. After the first tap there occurs complete contraction. As the tapping continues this state of contraction is maintained for several seconds, sometimes even for from one half to one minute; but sooner or later, in spite of continuous stimulation, the Hydra slowly expands. When it has reached its normal state of expansion it remains in that position as long as the stimulus is not increased, or even when it is slightly increased. Thus Hydra soon becomes used to a slight non-localised mechanical stimulus recurring at frequent intervals, and no longer responds to it. If the increase in

the force of the taps becomes very marked, or if by the motion of the water the Hydra is thrown against any solid surface, contraction recurs.

When in its natural surroundings, whether in stagnant or running water, Hydra is exposed to just such a rapid succession of stimuli, due to the constant motion of the water. If it were not for its power of acclimatisation to such a succession the Hydra would necessarily be constantly in a state of contraction. The response on increasing the force of the blows may have its biological significance as a protection against being washed away by any sudden increase in the motion of the water.

If the interval between stimuli is considerably increased so as to allow the Hydra to expand fully after each contraction, the tap being given the moment expansion ceases, the result is a different one. There is, in this case, no change whatever in the reaction after repeated stimulation. The course of events after the fiftieth tap is no different from that after the first; after each stimulation there is a contraction, followed shortly by re-expansion. Thus we get a different result so far as acclimatisation to the stimulus is concerned, depending on whether the stimuli are repeated rapidly or only after a considerable interval. In the lateral case one stimulation has evidently no effect on the response to a succeeding one. Recovery from the acclimatising effect must, therefore, be very rapid.

Localised Mechanical Stimuli.—In order to apply localised mechanical stimuli I prepared capillary glass rods, attached by means of sealing wax to larger glass rods as handles. With such a rod it was very easy to touch with any desirable force any part of the Hydra without producing any movement in the water such as in itself might cause a contraction.

A Hydra touched with such a rod will, of course, contract, provided the blow is not too light. This contraction is usually so sudden that no details of the process can be observed. In some cases the body does not at once com-

pletely contract; instead, it partly contracts, remains at rest a few seconds, then, without additional stimulation, contracts further, and so on, repeating the process three or four times before contraction is complete.

Of variation in sensitivity between individual Hydras I have already spoken. I attempted to discover whether there was any such variation between various parts of an individual. There is one difficulty in the way of such an attempt. Even after considerable practice it is almost impossible to give two successive stimuli of exactly the same magnitude. Perhaps slight differences in sensitivity would be thus obscured; but I am convinced that so large a number of experiments as were made would eliminate this source of error. I therefore feel justified in saying that all parts of Hydra are about equally sensitive. An individual Hydra will give practically the same response after each of many stimulations of approximately equal strength. This is true whether the successive stimuli are applied to the same region of the body or to different regions of the body, foot, hypostome, or tentacles.

Next the effect of stimuli of different intensities was tested when applied to the same part of the organism. Of course, very sensitive specimens completely contract after even an excessively weak stimulus. But in most cases there is a variation in the reaction parallel to the variation in the stimulus. This is not in accord with Marshall, who states (loc. cit.) that in response to an external stimulus both body and tentacles always contract.

Remembering that "weak" and "strong" are relative terms only, the reactions may be perhaps classified as follows:

A. Stimulation of body:
   1. Weak: body partly contracts.
   2. Medium: body completely contracts.
   3. Strong: body and tentacles contract.

B. Stimulation of a tentacle:
   1. Weak: tentacle stimulated contracts.
   2. Medium: all tentacles contract.
   3. Strong: tentacles and body contract.

Minor deviations are numerous, but all fall readily into the above scheme. One Hydra, for instance, was stimulated at the tip of a tentacle while body and tentacles were expanding. The tentacle stimulated contracted sharply, but the rest of the organism kept on expanding. It was only after this tentacle, now contracted, was stimulated a number of times that the whole Hydra contracted. After a short period of rest it expanded again. The stimulation was repeated in the same manner as before, and the same result was obtained step by step. It is a fact worth noting here that in most Hydras an exact repetition of a stimulus, after an interval of several minutes, reproduces the same sequence of events as at first.

In another Hydra I could make the tentacles contract one by one by means of stimulation at their tips, until all were contracted. The body in the meanwhile remained expanded, and contracted only after the last tentacle to contract has been stimulated a number of times. In other specimens repeated stimulation of one tentacle first caused the contraction of this one, then of some other, or several others, until all were contracted. Finally, the body also contracted. There is seemingly no constant relation between the tentacle stimulated and the one immediately succeeding it in contraction. This latter is sometimes the one standing next to the one stimulated, but by no means always. Quite as frequently it is the one opposite, or any other one of the circle. There occurred cases where the body contracted simultaneously with the last arms to contract; but in no case, where the stimulus was applied to a tentacle, did the body contract and leave some of the tentacles expanded; that is, a stimulus applied to one tentacle did not radiate to the body without also radiating to all the other tentacles. It did often radiate to these tentacles without reaching the body. This indicates that there is a particularly intimate connection for the transmission of stimuli between the individual tentacles; such connection would necessarily be through the hypostome. This is, of course, far from assuming that these parts are more sensitive

to external stimuli. I have already stated that this is not the case as regards mechanical stimuli.

So far I have spoken chiefly of stimulation of the tentacles. It is also possible by careful slight stimulation on the body to cause the latter to contract without contraction of the tentacles. It is not, however, so frequent a result as contraction of the tentacles without contraction of the body. This is for the reason, as far as I can make out, that the violent contractions of a mass, relatively so large as the body, results in a recoil which is often strong enough to affect the tentacles. Their contraction in such a case is caused by this secondary stimulus, without transmission to them of the primary one.

Sometimes the body, after a very slight stimulus, contracts only partially, and then immediately expands again. At other times, when it contracts completely, the tentacles will remain expanded for from twelve to thirty seconds or more and then suddenly contract.

In Hydra with buds it is possible to stimulate the body of the parent so as to cause its contraction without contraction of the bud; or the bud can be stimulated and caused to contract without contraction of the parent. But here also the recoil from the contraction of one part interferes with any fine gradations in the response.

It is to be noticed that in all these results there is no indication of any orientation movement on the part of the Hydra. The organism does not move definitely toward or away from the source of stimulation. No matter where the stimulus is applied the single response is simply a contraction, partial or complete. Now, as the foot is attached to the substratum, it definitely fixes the direction in which contraction will move the Hydra as a whole. Contraction causes a moving of the mass of the Hydra towards the foot. The contraction so fixed, there can of course not be any fixed relation between it and the place at which the stimulus is applied; for no matter where the stimulus acts, the direction of the contraction is always the same. In hundreds of experiments I have not seen a single case where Hydra showed any bending either of

body or of tentacles definitely toward or away from the source of mechanical stimulation.

Considerable importance attached to this comparatively simple point. It is at present quite frequently assumed that all organisms respond to stimuli having hedonic value, by either moving toward or away from the source of stimulation. Of sessile organisms it is similarly stated that they expand toward or contract away from the stimulus. So Professor Baldwin ('97, pp. 198–9) says: "All organisms behave in two great and opposite ways towards stimulations; they approach them, or they recede from them. Creatures which move as a whole move towards some kinds of stimulations, and recede from others. Creatures which are fixed in their habitat expand towards certain stimulations and contract away from others." . . . . "The stimulations which the organism tends toward are those which heighten its vitality, which give it pleasure; and those from which it draws back are those whose effect upon it is the contrary—the damaging, the painful ones."

If we try to apply this statement, as far as it concerns contraction, to the response of Hydra to a mechanical stimulus, we find that it goes beyond the facts. Hydra does indeed contract after such stimulation. But this contraction is not necessarily a movement away from the stimulus. It is such if the stimulus is applied to hypostome or tentacles. It is not such if the stimulus acts near the foot. In this case, in fact, the body comes as a whole nearer to the source of stimulation than it was when expanded. This is true also of a tentacle which contracts when stimulated near its base. As a whole the tentacle approaches the source of stimulation rather than moving away from it. It may be objected that the stimuli given in the laboratory represent artificial conditions, and that in nature the contraction of Hydra probably draws it away from the painful stimulus. But to make this objection allowable it must first be shown that harmful stimuli are more apt to reach the hypostome than the foot. At present I see no reason for believing that such is the case.

It seems to me that Richet, as quoted by Baldwin (l. c., p. 178), has expressed the probable object of contractions much better when he says, "There takes place a series of general movements of flexion, as if the animal wished to make itself smaller, and to offer less surface to the pain. . . . ." This is exactly the result we get in Hydra. As it contracts it becomes more nearly spherical, and so reduces the size of its exposed surface. It is in this reduction of exposed surface, and the consequent reduction of the chances of being hit, that the adaptive value of contraction in Hydra really lies. Perhaps the closer crowding of cnidoblasts, consequent on such reduction of surface, also plays a part.

What will happen if a localised mechanical stimulus is repeated at regular intervals? A Hydra viridis was stimulated so as to contract. It was then allowed to expand again, but the moment expansion was complete the stimulus was repeated, and so on for several hours. Two questions are here of special interest: first, does repetition of the stimulus cause Hydra to contract less readily; and second, has such repetition any effect on the subsequent re-expansion, either as to rapidity or the occurrence of any orientation movement? Both questions are answered decidedly in the negative as far as stimulation at longer intervals is concerned. Here, as with non-localised stimulation, the response after many stimulations did not change. The contraction was as rapid and as complete after the fiftieth stimulation as after the first.

Re-expansion also was not changed in character. As to the direction of such expansion one might perhaps expect that in the course of time it would be in a direction away from the side from which the stimulus was applied. But no such thing occurred. As after spontaneous contraction, so here re-expansion was toward a different direction after each contraction. But this change in direction could not be referred in any way to the direction from which stimulation came. The re-expansion was as often toward the stimulus as away from it, and equally often it was at right or oblique angles to the direction of stimulation. Such repeated stimu-

lation at longer intervals does not, therefore, cause any change in the reaction. In other words, we do not here see any evidence of acclimatisation or memory.

A second method of studying the effects of repeated stimulation varied from the first only in the length of the interval between stimuli. Instead of allowing the Hydra to expand after each stimulation the stimulus was applied at intervals of about one second. There results of course a contraction. The Hydra remains contracted for from one half to one minute. Then, in spite of continued stimulation, it slowly expands, and does not again contract unless, as often happens, the intensity of the stimulus is accidentally increased. If, on the other hand, the blows are kept up without any increase in intensity, one of two things happens:

(1) In many cases the Hydra now acts as if no stimulation were present; it entirely ignores the blows of the rod.

(2) In a minority of cases the result is decidedly different. The Hydra slowly bends its body to one side until its expanded tentacles touch the glass at some distance from the foot. They then attach themselves and contract. The foot loosens its hold, and the body of the Hydra contracts. But the body immediately re-expands and bends over, until the foot touches the glass close beside the tentacles. The foot now reattached itself, the tentacles loosen their hold, and the body straightens out. The Hydra thus again occupies its normal position, but at some distance from the place where it was subject to stimulation. Its further movements are those of a normal Hydra. As to the direction towards which Hydra travels in the "escape," it has again no relation to the direction from which stimulation comes. To resort again to the points of the compass as convenient symbols of direction let us suppose the glass rod to stimulate the Hydra on the west side. I have seen such a Hydra escape toward the east. But I have seen others escape in almost all of the other directions. Some even bent toward the west, over the rod that stimulated them, and escaped in that direction. Here again, therefore, we have in no sense an orientation.

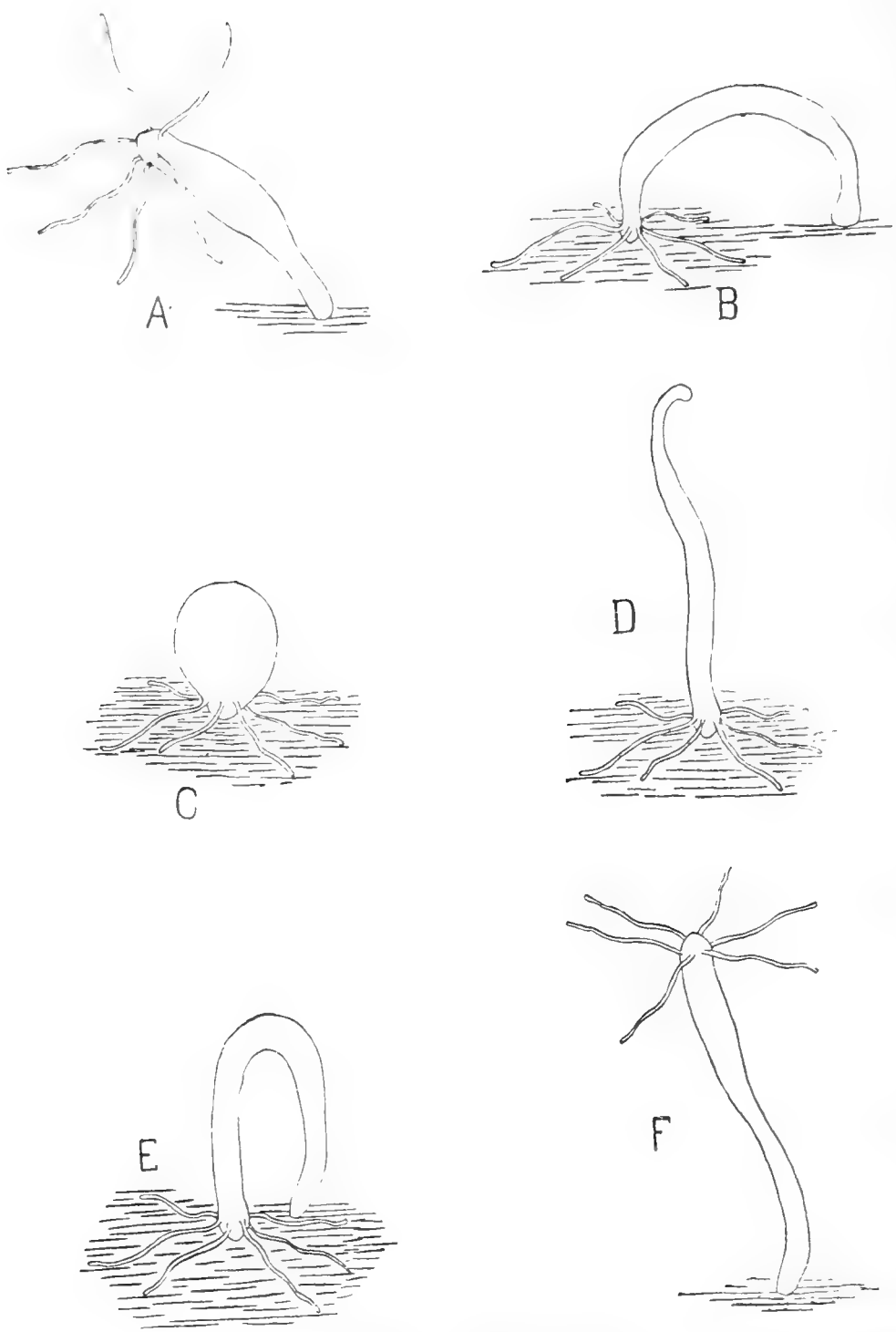

FIG. 3.—Common method of travel in Hydra. A. Body bending toward substratum. B. Tentacles attaching themselves. C. Foot loosened and body contracted. D. Body re-extended, foot end beginning to bend over. E. Foot attaching itself near hypostome. F. Upright position regained.

As to the mechanism of this "escape" movement it will be readily recognised that it is identical with the mechanism ordinarily employed by Hydra to move from place to place, as shown in Fig. 3. The important point is that here on repeated stimulation there is at first a simple contraction, then comes a period where apparently there is some sort of acclimatisation to the stimulus which now has apparently no effect. However, this is only apparently so, for there may soon follow the third stage in which the Hydra responds to the stimulus by a movement entirely different and even directly opposed to the first response by contraction. This final movement is furthermore one of very considerable complexity.

Jennings ('02) has found in Stentor and Vorticella a very similar modification of reactions due to repetition of stimulation. In Stentor roeselii, in response to continued stimulation by powdered carmine, there is the following sequence: bending away from source of stimulation, reversal of ciliary current, contraction, and finally abandonment of its tube. In Stentor coeruleus the results are similar. When continuously stimulated by means of some solid, like powdered carmine, this form responds at first by bending into a new position; on continuance of stimulation it reverses the ciliary current, and thus repeats its first manœuvre; this is followed by contraction, and finally by loosening its hold on the substratum and swimming away.

These responses result from stimulation by carmine powder, which stimulates in part chemically. If a purely mechanical stimulus be used Stentor coeruleus at first contracts after a single blow, but subsequently it may require as many as forty. But eventually this form always loosens its hold on the substratum and swims away. In particular sensitive individuals this last movement is resorted to at the first blow from a glass rod, but this is the exception. Stentor roeselii, however, as well as Vorticella and some other forms, show true acclimatisation, and never break away as the result of a purely mechanical stimulation.

Jennings (l. c., pp. 49–51) discusses at length the possible explanation for the failure on part of an organism to react to a stimulus to which at first it responds very readily. The three possibilities he mentions are motor fatigue, sensory fatigue, and a third unknown element. The facts concerning Hydra that bear on this matter fully support Jennings' contention. Motor fatigue is entirely out of the question, for I have been able, under proper conditions, to keep Hydra contracting continuously for as long as three hours, at the end of which time it responded as readily as at the beginning. Furthermore, we have seen that after long repeated stimulation at such frequent intervals as to bar any possibility of recovery from fatigue, Hydra finally undertakes the very complicated "escape" movement. This, in itself, involves a large amount of work, and makes explanation by motor fatigue impossible.

Further, this last-mentioned "escape" movement proves, with equal force, that the stimulus is still perceived, otherwise there could be no reaction. This being the case, the explanation by sensory fatigue is clearly inadmissible. There remains only the third possibility. We may suppose that the stimulus which causes a contraction at the same time affects the physiological condition of the organism in such a way that the limen for that particular character of stimulation is raised. If part of the energy involved in the stimulus comes to act on the chemical constituents of the organism this may well cause a change in the character of these constituents. Irritability must depend largely on the chemical character of these constituents; this, it seems to me, is well shown by the action of narcotics and anæsthetics. Therefore, a chemical change in body constituents would necessarily involve a change in irritability, and so a change in the readiness of response to stimulation. These considerations may very well explain the lack of response after repeated stimulations.

The return of the Hydra to a position of semi-extension, after a contraction reaction, is apparently simply a return to the position of rest. Zoja (l. c.) found that Hydra, anæsthe-

tised by chloroform or ether, always assumed this position of semi-extension.

There still remains the very involved "escape" movement. It will be useful to compare this with the reactions of Paramecium toward stimuli of various kinds as studied by Jennings. Paramecium responds by stopping its spiral movement, jerking backwards, swinging toward the aboral side, and finally moving forward again. If it then comes in contact with the source of stimulation again it goes through the whole manœuvre again, until finally its forward movement carries it out of the region of stimulation. As far as is now known this method of reaction is never varied as a result of experience. That escape is finally effected depends entirely upon the element of chance involved. The movement, as such, bears no relation to the source of stimulation; it bears a very definite relation to the structure of the Paramecium.

The mere contraction of Hydra in response to a mechanical stimulus is a reaction quite parallel to this. It also has no relation to the direction from which stimulation comes, while it has a fixed relation to the structure of the Hydra. There is, however, a difference. If the Paramecium does not succeed in escaping from the stimulus at the first trial, it may do so at a second or any subsequent attempt. If the Hydra does not escape by the first contraction it will not escape by a subsequent one, for its sessile mode of life precludes the element of chance involved in the movements of a free swimming form like Paramecium. Hydra, however, does not continue to respond indefinitely by contraction, but resorts, after a short time, to another method not a necessary consequence of the first, and by this second method accomplishes its purpose. This second movement also has no strict relation to the direction from which the stimulation proceeds. Nevertheless, it always succeeds in removing the Hydra from the influence of the stimulus. Having no fixed relation to the stimulus the idea arises that it may have a definite relation to some structural feature of the Hydra. But evidence

on this point is lacking, Hydra being purely radiate, as far as is known.

On the whole Hydra, in its manner of reaction toward mechanical stimuli, is a close parallel of Stentor and Vorticella. Jennings ('02), in his paper on these forms, has discussed, at some length, the psychological questions involved. This discussion applies with equal force to the reactions of Hydra.

Rheotropism.

In order to determine whether Hydra reacted in any definite way to a current of water a very simple apparatus was constructed by taking a glass tube eighteen inches long and about one and a half inches inside diameter. A small hole was made into one side near the middle for the introduction of the animals. The tube was placed horizontally, and so arranged that water flowed into it over a dam made of a bisected cork, and flowed out at the other end over a similar obstruction. After a number of Hydra were introduced the current of water was turned on. It could be increased or diminished by regulation of the amount of water supplied, and especially by tilting the tube. In this apparatus I had Hydra under observation for a number of days, but there was absolutely no sign of response to the currrent. The current was certainly much stronger than that to which the Hydra is ordinarily exposed in nature. Yet there was no travelling either up or down stream, nor any curvature of the body with or against the current. There is thus no evidence of rheotropism in Hydra.

Righting Reaction.

Hydra usually has its foot attached to the substratum, while body and tentacles, moderately extended, project out

into the water. In Hydra viridis, as well as in the other forms, the tentacles frequently attach themselves to the substratum and assist in movement. But they remain so attached only for very brief periods. The foot, on the other hand, is seldom detached. Loeb ('91) has shown that Cerianthus,

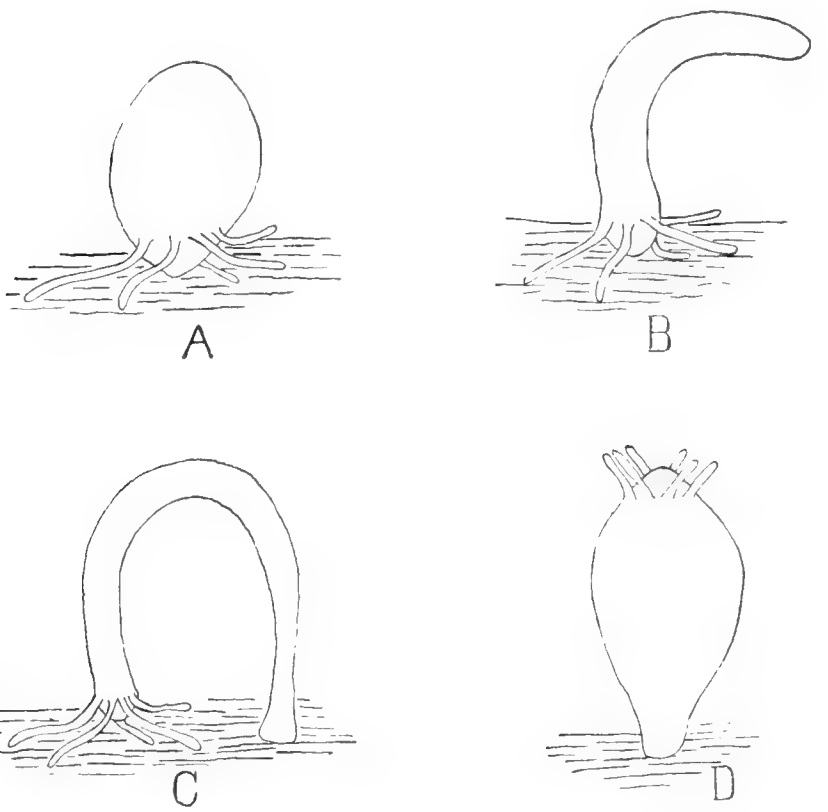

FIG. 4.—Righting reaction of Hydra viridis. A. Hydra placed in reversed position, tentacles attached to substratum. B. Body extending, foot end bending over. C. Foot attached, tentacles ready to loosen. D. Upright position regained.

placed in an unnatural position, will strive successfully even under difficulties to place itself in such a position that its long axis is vertical, with head up, and foot attached to the substratum. In Hydra a simple experiment shows that a similar righting reaction occurs. A green Hydra, strongly contracted, is taken and placed so that it rests on the bottom of a watchglass on its tentacles, which act like the legs of a

stool. The foot of the animal extends straight up into the water. Almost immediately the body begins to extend. When about half extended it bends to one side until the foot touches the glass and attaches itself. Sometimes the tentacles loosen before the foot is attached and the Hydra simply tumbles over. But more commonly the entire action is an active one of orientation, not a passive tumbling over. I have tried to represent the process in Fig. 4.

We have here, then, a process comparable to that observed by Loeb in Cerianthus, but not quite so complicated. Cerianthus seeks not only contact for its foot but a vertical position. In Hydra thigmotaxis alone is involved, for it can be easily observed that Hydra, normally, have their long axes disposed at all possible angles to the force of gravity.

Reactions to Chemical Stimuli.

Non-localised.—To any solution in which Hydra is immersed it responds, if the solution be strong enough to affect it at all, by a general contraction. Only in rare cases is another reaction produced; these will be discussed in the account of the food reactions. When contraction does occur the body remains contracted for a considerable time, then slowly begins to extend. But this extension never goes very far before contraction again occurs. This continues until the animal perishes, usually in a comparatively short time.

Localised.—The method of applying localised chemical stimuli was as follows:—The tip of a capillary glass tube was pressed into the finely powdered chemical. In this way a considerable amount of the chemical was forced up into the tube. If the end of the tube was then placed under water the water dissolved the chemical slowly, and the solution gradually diffused from the mouth of the tube. If this mouth was placed very close to a Hydra the diffusion cloud would

strike at first a very limited area on the surface of the body. In other words, the stimuli was distinctly localised. A number of chemicals were experimented with in this manner including citric acid, acetic acid, sodium chloride, sodium carbonate, potassium bichromate, methylene blue, and others. The action of all was precisely the same. I found, however, that methyl green was most convenient to handle, on account of the colour, by means of which the limits of the diffusing cloud, were easily recognised, and because it gave the most certain results. I shall describe as a type, therefore, the effects obtained by the use of this substance. That the results obtained were due to chemical action, and not to mechanical agitation is shown by the fact that no effect was produced by streams of distilled water or of an excessively weak solution of methyl green used in the same manner.

When a cloud of methyl green is allowed to strike Hydra a little above the foot, in a few seconds there is a flexion of the body at the point where the chemical touches it. The body bends over slowly, and this bending is toward the side on which the chemical acts. In some cases there is also a separate bending just below the hypostome, toward the same side. This latter peculiar movement gives the appearance of the presence of a neck region. The same movement has been observed by Pearl ('01) under the influence of the electric current, and it can also be observed as occurring in apparently unstimulated Hydra. As the methyl green spreads and affects a larger area on the surface of the Hydra complete contraction results.

If the methyl green is similarly brought near to the middle of an expanded tentacle the tentacle flexes at this point in the direction from which the chemical approaches it. Then, as it enters the denser cloud of stain, it contracts. The tentacles are decidedly more sensitive to the action of the chemicals than the rest of the body. Usually the tentacle flexes before the visible green cloud has reached it, indicating that the edges of this cloud, containing yet too little stain to be visibly coloured, are still concentrated enough to produce

the reaction. This never is the case when the body is the part stimulated.

If the tube is placed inside the circle of tentacles, so that the cloud first reaches the surface of the hypostome, the tentacles simultaneously sway inward, then contract.

Here we have then a definite orientation reaction toward a chemical stimulus. It is important that we understand just how it comes about. If after the application of such a localised stimulus we carefully remove the methyl green that has gone into solution by means of a pipette, the Hydra will soon begin to expand. But now it can be noticed that in the region affected by the stain there is no expansion, and the ectoderm in this region is seen to be permanently stained. Often there is no return of mobility in this region, even after some hours, the cells in such cases being probably dead. On account of this local absence of expansion the body, as soon as it begins to expand, also begins to flex again toward the same side as it did before under the influence of the stimulus.

The direct result then of such local chemical stimulation is a contraction of those ectoderm cells that come in direct contact with the chemical. This local contraction necessarily causes the body (or tentacle) to flex at the point affected, and to flex toward the side from which the stimulus acted. But only injurious chemicals cause this reaction, and by it the Hydra is carried into a destructive solution. This bending of the body is, therefore, anything rather than adaptive in nature. It is probable that such strongly localised chemical stimuli of a destructive nature play no part in the normal life of Hydra. The reaction, therefore, forms no part of the normal behaviour, but is more or less pathological in character. It recalls the false tropisms in plants, more especially false traumatropism. Ciesielski ('72) found that when a root was severely injured on one side at some point lying within the growing zone there was produced a curvature at this point, the curving being toward the side injured. If, on the other hand, the injury was only slight a curvature also resulted, but in this case it was away from the side injured. The

latter result is a true response to stimulus; the former is a purely mechanical effect, due to the fact that the severely injured cells ceased growing and the continued growth on the opposite side forced the root to curve toward the injured side. The response to a chemical in Hydra is probably a mechanical result of this kind, except that in place of growth it is movement that is checked.

Food Reactions.

The process of taking in food as it occurs in Hydra has, as far as I know, been described in detail only by Hartog ('80), although Trembley has a long general discussion of it. Hartog's note on the subject was unknown to me until after I had studied the process myself and written out a description that coincides in large part with his.

If a Hydra that has been kept without food for a week or ten days has a bit of raw meat placed on or very near one of its tentacles the course of events is about as follows:—The tentacle first touching the meat fastens itself to it, apparently by means of some secretion, and then contracts. The other tentacles begin rather active movements, which, however, show little correlation. Nevertheless, soon all the tentacles find their way to the meat, become fastened to it, and contract. There is, however, so little definiteness in all these movements that the meat often falls away from the tentacles. In such cases the Hydra makes no very great effort to find it again. Vague movements of the tentacles may continue for some time; sometimes they even strike simultaneously toward the hypostome, as if clutching for something.

In other cases, however, the food is by the movement of the tentacles brought to the hypostome. As soon as this is touched by the meat, sometimes even some seconds before, the mouth begins to open, and its edges fasten to the meat. Immediately the tentacles loosen their hold and swing away from the mouth. They play no further part in the swallowing. It is usually stated that they push the food into the mouth,

but this is not at all the case. The actual process of swallowing depends entirely on the activity of the tissue of the hypostome and body. Occasionally a tentacle remains attached to a food particle after the mouth has opened, and in some cases the tentacle is even drawn into the food cavity with the food. But in all such cases which have come under my notice it could be seen that the tentacle was passively pulled along, having by its nematocysts, or in some other way, become attached to the food, and being unable to release its hold.

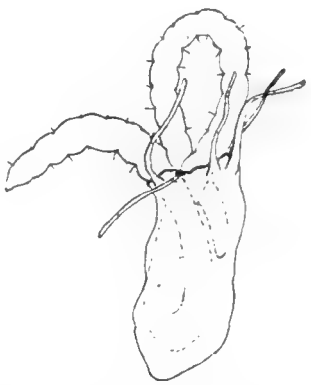

FIG. 5.—Hydra grisea, with partially ingested annelid. (Camera lucida drawing.)

The food is now slowly drawn into the body cavity, but at the same time another movement takes place. The hypostome slowly draws itself upwards over the food particle. This peculiar action is very well compared by Hartog (l. c.) to the method in which a snake gets outside its prey, or in which an automatic stocking might stretch itself onto the foot and leg. The food once well within the cavity the mouth closes. Now the body-wall expands for some distance in front of the particle, while it strongly contracts behind it. And so the food is forced to the lower portion of the food cavity.

Both cilia and pseudopodia have been observed on the ectodermal cells of Hydra, and it is commonly stated that it is the former that are chiefly concerned in the act of swallowing. But it is difficult to see how cilia could have enough strength to play any part in the swallowing of entire entomo-

straca, annelids, insect larvæ, and the like, which form the ordinary food of Hydra. The difficulty increases in such a case as came to my notice recently, where a Hydra grisea made an attempt, partially successful, to swallow an annelid fully fifty times its own bulk (Fig. 5). The extent to which the tissues of the Hydra were stretched during this performance is almost beyond belief. Another Hydra from the same dish attempted the same gastronomic feat on a leech (Clepsine) certainly five hundred times its own bulk. Needless to say this one did not succeed. It seems to me we must assign the leading rôle in swallowing to pseudopodia. I hope, however, to obtain direct evidence on this point from sections before long.

The food having reached the lower portion of the digestive tract, rests here until digestion is complete, except for the fact that it is churned up and down the cavity more or less as the Hydra expands and contracts. Digestion completed, the insoluble residue is discarded through the mouth. This ejection I have seen performed several times, and in every case the food was forced out by a very sudden squirt that threw the débris to some distance.

During these experiments it was soon noticed that Hydras would not always attempt to capture food that was offered. Hydras taken from a dish in which food was abundant were commonly quite indifferent to any food offered them, no matter what its character. The same Hydra were then kept in a dish of filtered water for a week, thus being deprived of all food material. At the end of this period they swallowed very readily any food offered them, the process of ingestion often being over in less than half a minute. Hydra, therefore, does not react to offered food at all times, but only after a period of abstinence. It is an intermittent, not a constant feeder. It sounds very simple to say that Hydra will not feed except when hungry. Nevertheless, a determination of the fact is not superfluous. The case is different for instance in Planaria; Pearl ('03, p. 668–9) found in these that the food-seeking reaction was not at all affected by conditions of

hunger, although the food taking was. In the protozoans even food taking (swallowing) is seemingly a continuous process. One sometimes sees Paramecia with bodies crowded with food material still steadily taking in more.

The Hydras used in subsequent work were therefore starved for a period of seven or eight days by keeping them in filtered water. There is a remarkable increase in activity in such Hydras. Their tentacles are in almost constant and rather rapid motion, and the body sways to and fro, expands and contracts, in a very nervous manner. Green Hydras stand starving very poorly, usually perishing in two or three days. I therefore used Hydra grisea for this part of the work. Its larger size gives it another advantage.

Let us now consider what factors in the food enable the Hydras to recognise it as such. It is evident that any stimulus coming from, say, a piece of meat must be either chemical or mechanical.

Let us consider the mechanical stimulus first. The result of experiments directed to this point could be foreseen. In studying the effect of mechanical stimuli several hundred Hydras, from many sources, were stimulated by prodding with glass rods on every part of the body, including hypostome and tentacles, and at all degrees of intensity. In no case did there result any reaction resembling in any way the movements concerned in feeding. To make sure, however, I tested Hydra that had been starved for from seven to ten days by placing minute bits of filter-paper on the hypostome. This paper had been soaked for several days in water from exactly the same source as that in which the Hydra were kept while starving. All possibility of chemical stimulation was thus removed. The result was as expected. There was no trace of a food reaction, and the paper soon rolled away from the animal. A mechanical stimulus alone, then, cannot call forth a food reaction.

Is the same thing true of a chemical stimulus? To test this I took Hydras, starved for seven or eight days, and placed them in small watch-glasses. The water was drawn

off from them as completely as possible, and filtered beef-tea made from beef extract was substituted. Of the chemicals in meat only the soluble ones can stimulate chemically, and these are well represented in beef extract. So if the stimulus producing a food reaction were purely chemical we might expect such a reaction here.

But there was no trace of it; the Hydras acted exactly as they did in ordinary water, except when the beef-tea was made too strong. In that case a Hydra contracted just as it does in the case of any other chemical in strong solution. So far as this evidence goes, therefore, a chemical stimulus alone is no more adequate to produce a food reaction than is a mechanical one. That this is not absolutely true we shall see in a moment.

Next, we may consider the effect of a combined stimulus. Some of the Hydras used in the experiment last mentioned were returned from the beef-tea to hydrant water. There were now presented to them minute pieces of filter-paper previously soaked in beef-tea. The result was very striking. As soon as the paper touched the tentacles these seized it and drew it toward the hypostome. As soon as the paper touched this the mouth opened, and the morsel was engulfed. Here, as always, no effort was made to turn the object in any way, so as to make swallowing it easier. The way it was presented was the way it went down. Such a piece of filter-paper is, however, usually anything but smooth. It has projecting fibres in many places, and these projections, after the paper was swallowed, caused the body wall of Hydra to bulge out in very grotesque fashion. The same experiment was repeated many times, always with the same result.

The food reaction can also be brought about by immersing a starved Hydra in beef-tea, and then stimulating the hypostome mechanically with a glass rod. The reaction does not, however, appear quite as constantly as in the previous experiments.

Thus we see that by properly combining a chemical stimulus with a mechanical one, the food reaction can be brought about in Hydra by an object which itself has no food value.

Having at hand some Hydras that had been without food for twelve days I started to repeat the same series of experiments on them. To my surprise they all responded with a typical food reaction when immersed in pure filtered beef-tea. Shortly after this was poured over them the tentacles began vigorous movements, which were, however, but little correlated. The mouth opened wide, and remained open for a minute or so, then it closed, and apparently some of the beef-tea had been taken in, for the body could be seen to be considerably expanded just back of the hypostome. In some cases this expansion even travelled backward. In fact there was, in every case, a very typical food reaction.

What is more, the same food reaction was secured by means of a solution of quinine. In this case, however, the quinine very strongly affected the entoderm after the mouth was opened, and the Hydra soon perished with its mouth wide open. Quinine is a strong poison to most of the lower invertebrates. So much the more remarkable is the fact that it should be able to call forth a food reaction.

These experiments then give us the evidence that there may be three factors concerned in the production of a food reaction. These factors are a state of hunger in the Hydra, a chemical stimulus, and a mechanical stimulus. The first two must be present to produce the reaction. The third is or is not necessary, depending upon the intensity of the first factor—hunger. A food reaction may thus be brought about in two ways: first, by the combined action of a chemical and a physical stimulus, in presence of a moderate degree of hunger; second, by a chemical stimulus alone, when the hunger has become intense.

While keeping Hydra under observation for a number of months it was noticed that ostracods formed no part of their food, though these crustacea were fairly numerous in the aquaria in which the Hydra were kept. It was further noticed that an ostracod could come freely in contact with Hydra at any time without calling forth any attempt at capturing it. This was the case even when the Hydra had been

starved for some time. A Cyclops presented to the same Hydra was captured and swallowed very quickly, as was a piece of raw beef.

Why does Hydra not capture and swallow ostracods just as it does others of the smaller crustacea? It seems to me the answer is not far to seek. Ostracods are enclosed in hard chitinous shells, which, as far as I have observed, are never opened more than a little way. The organic fluids given off by the animal therefore escape very slowly beyond the confines of the shell. Hence the minute chemical stimulus given by an ostracod as it comes into contact with the Hydra is inadequate; but adequate chemical stimulation is, in all cases, a prerequisite of the food reaction, and so the ostracod, giving only a mechanical impulse, is not recognised as food. If this theory is correct, then we ought to be able to cause the Hydra to swallow the ostracod, by bringing to bear a chemical stimulus while simultaneously presenting the ostracod. This is easily done by crushing the ostracod slightly, so that some of the juices of the body freely flow out. If such an ostracod is presented to a starved Hydra the rapidity with which it disappears into the food cavity is little short of marvellous.

Nematocyst Discharge.

As is well known Hydra, like most cœlenterates, carries in its ectoderm a great number of cnidoblasts. They are fairly plentiful over most of the body, but occur most numerously on the tentacles, where they form small tubercle-like masses. They seem to serve chiefly for the capture of prey, though secondarily they may also form a means of protection against enemies.

There has been considerable controversy as to the nature of the action of the nematocysts on the organism at which they are discharged. Various authors (for instance, Schneider, '90) claim that the usual supposition that the nematocysts

penetrate the epidermis of the prey is incorrect. They argue that the nature of the nematocyst is too fragile to permit of such a result. The nematocysts, according to their views, merely adhere to the outside of the captured animal. Grenacher ('95) is, as far as I know, the only one who has published a direct observation on this subject, reproducing from memory a drawing of an observation made many years previously. It represents a single nematocyst that has penetrated the cuticle of a Culex larva. Opportunities for such observations are not so frequent as might be supposed. Professor Jennings was so fortunate, however, as to procure

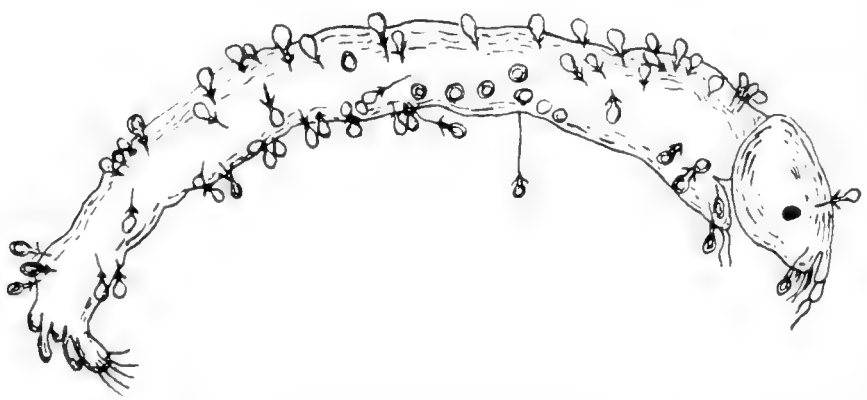

FIG. 6.—Insect larva pierced with nematocysts. (Copied from a drawing by Mrs. Jennings.)

a specimen that settles the point for Hydra very conclusively. The victim, a fly larva, is thickly covered with Hydra nematocysts, and these can be plainly seen to penetrate the skin, and project into the interior of the larva (Fig. 6). Not only the point, but, in some cases, the lateral barbs also have penetrated. The specimen was taken from among the tentacles of a Hydra just as the latter was preparing to swallow it.

The effect of the nematocysts is almost instantaneous paralysis or even death of the animal attacked. It seems, moreover, to be effective on Hydra itself. On one occasion, seeing a Hydra grisea with its mouth widely open, I quickly thrust a second Hydra into it. The first one made an attempt to swallow it, but did not get very far, for the victim began

a very profuse discharge of nematocysts. The first Hydra reciprocated, and in a very short time both Hydra were apparently dead.

As far as my observations go the nematocysts are not always necessary for paralysing prey. Apparently this is sometimes brought about by some purely fluid discharge from the tentacles. In larger specimens of Hydra grisea the discharged nematocysts can easily be seen under the Braus-Drüner microscope. Yet I have often observed, with such a microscope, that small crustacea, such as Cyclops, were paralysed when approaching too near the tentacles, without any discharge of nematocysts. At least, very close search, even with a microscope of higher power, disclosed none. It may be said that what occurred here is simply the death-feigning so common in the lower crustacea, but that is not the case. Death-feigning lasts only a very short time. In the phenomenon under consideration the animal remains motionless for many minutes, and then, provided the Hydra has been removed, or, for some reason, does not attempt to swallow its prey, motion slowly and gradually returns.

The structure of the cnidoblast, and especially the protruding cnidocil, suggest a direct mechanical arrangement for its discharge. Schulze ('71) suggests such an explanation for these discharges. He believed that a pressure from without on the cnidocil would directly disturb the mechanical equilibrium in the cnidoblast, and so cause a discharge. After the muscular and nervous nature of certain cells in Hydra was recognised the explanations offered took on a more physiological character; according to Chun ('93) there was involved a long passage of the stimulus through distinct ganglia and nerve fibres; but all explanation, as far as I know, hold to the idea that the cnidocil serves the mechanical function of a trigger.

Zoja (l. c.) found that he could touch the cnidocils repeatedly and rudely without getting a discharge of nematocysts, but he did not draw any conclusions from this fact. R. v. Lendenfeld ('83), working with actinians, found that the ten-

tacles discharged their nematocysts when touched by a particle of some digestible substance, but not when sand was allowed to fall on them. Although he found the same thing true of an isolated tentacle he expressed the opinion that nematocyst discharge depended on the will of the animal.

I tried similar experiments with a great many Hydras of various species, and in no case was I able to secure a discharge of nematocysts by mechanical stimulation alone. Large specimens of Hydra grisea, in which the nematocysts could easily be recognised under the Brauns-Drüner microscope, were stimulated by means of a capillary glass rod. The tip of the rod was moved over the body in all directions, often with pressure enough to produce a wound. But even after five or ten minutes of such treatment no discharge of nematocysts results. There is such a discharge when Hydra is crushed under a cover glass. This discharge is due, however, to the direct pressure brought to bear on the cnidoblasts, and has no bearing on the normal reaction, as it is in no sense a vital phenomenon. It is clear, therefore, that mechanical stimuli are not adequate for nematocyst discharge. The cnidocils must, therefore, serve a function different from that suggested by their common name of "trigger."

Chemical stimuli was next resorted to. Various chemicals were used, such as acetic acid, methylene blue, citric acid, and methyl green. It was found that all of these, if in solutions of proper strength, would cause nematocyst discharge. Even beef tea, when strong enough, had the same effect. The most certain in action, however, and, on account of its colour, the easiest to observe, was methyl green. If a small amount of solid methyl green was applied to a Hydra in the manner previously described (pp. 603 to 606) there was always a discharge of nematocysts, provided the solution of the stain was not too dilute when it reached the Hydra. This discharge is absolutely limited to the area directly touched by the stain. After the stain had acted a short time the surplus was removed by a pipette or the Hydra was placed in another watch-glass. It was then easily seen that the ectoderm cells

over the area directly touched by the stain-cloud were stained green, and that nematocyts were discharged only over the area so stained.

Similar results are obtained by experimenting with tentacles cut off from the body. A tentacle can thus be cut at any place without causing any great discharge of nematocysts. If such a tentacle is then stimulated with methyl green it will discharge nematocysts profusely, just as it would have done had it remained attached to the body. The discharge is restricted absolutely to the region touched by the solution of the chemical.

The discharge of nematocysts depends then entirely upon chemical stimulation. The action of the stimulus is probably a very direct one on the protoplasm of the cnidoblast, for if there were involved a nervous mechanism of such complexity as Chun (l. c.) supposes, we could reasonably expect the discharge to reach beyond so strictly limited an area. It is also obvious that the cnidocil can only serve a sensory function, as was suggested by Schneider ('90). The results with isolated tentacles certainly dispose of v. Lendenfeld's theory of the discharge as controlled by the will of the animal.

To my teacher and friend, Professor H. S. Jennings, I am deeply indebted for constant aid and encouragement in this work. It was undertaken at his suggestion and carried out in his laboratory. To Professor Reighard I am grateful for more than ordinary courtesies extended to me while in the laboratories in his charge.

Summary.

The principal points that I have attempted to bring out in this paper are:

1. An undisturbed Hydra does not remain motionless, but contracts at fairly regular intervals. After contraction it expands in such a way as to occupy a different position from that previously occupied.

2. Hydra has only one form of response to a single mechanical stimulation, localised or non-localised; this response is by contraction, more or less complete, depending on the intensity of the stimulus. Such contraction is not necessarily toward or away from the stimulus.

3. A non-localised stimulus, repeated as soon as Hydra has regained the expanded stage, causes no change in the response, contraction resulting after each stimulation. The same thing holds true of a localised stimulus applied in a similar manner.

4. If a non-localised mechanical stimulus is repeated at very brief intervals, say one second, acclimatisation is soon affected, and the Hydra no longer responds.

5. A localised stimulus applied at such brief intervals brings about at first an apparent acclimatisation. This is soon followed in many cases by the complicated "escape" movement, the Hydra moving away from the region where stimulation occurs. This shows that the physiological condition of the animal has been changed, so that to the same stimulus under the same external conditions it now gives a reaction different from that given at first.

6. Hydra shows no orientation movements in response to stimulation by a current of water.

7. Hydra normally has its foot attached to the sub-stratum. If the foot is detached the animal performs active movements directed toward restoring the normal condition. Geotaxis plays no part in this reaction.

8. Non-localised chemical stimuli cause general contraction. An exception is found in certain food reactions.

9. A strong localised chemical stimulus causes a bending of the body or tentacles, as the case may be, toward the side stimulated. Such bending is caused by the contraction of the ecto derm cells directly affected by the chemical. The result is not adaptive, as it carries the body or tentacles into the region where it is most injured.

10. Hydra reacts to food only after a period of hunger.

11. In the presence of a moderate state of hunger it

requires a combination of a chemical and a mechanical stimulus to produce a food reaction. If starvation is extreme a chemical stimulus alone suffices.

12. A mechanical stimulus will not produce a discharge of nematocysts; a chemical stimulus will.

13. The action of the chemical is probably quite direct, not involving the nervous system. Nematocyst discharge is restricted absolutely to the area touched directly by the chemical.

14. The nematocysts can, and do, pierce the epidermis of the prey at which they are discharged.

15. Hydra seems to be able to paralyse prey without discharging nematocysts.

Bibliography.

Baker, H.—1743. 'Essays on the Natural History of the Polypes,' London, 1743.

Baldwin, J. Mark.—'97. 'Mental Development in the Child and the Race: Methods and Processes,' New York, 1897 (Second Edition).

Ciesielski, T.—'72. 'Beiträge zur Biologie der Pflanzen (Cohn),' Bd. i, zweites Heft., p. 1.

Chun, Carl.—'93. "Die Canarischen Siphonophoren. II. Monophyiden," 'Abhandl. d. Senkenb. Nat. Gesell.,' 1893.

Grenacher, H.—'95. 'Zool. Anzeiger,' Bd. viii, 1895, p. 310.

Hartog, M. M.—'80. 'Quart. Journ. Micr. Sci.' (N. S.), vol. xx, p. 248, 1880 a. Same volume and page.

Jennings, H. S.—'97. 'Journal of Physiology,' vol. xxi, pp. 258—322. 1902. 'American Journal of Physiology,' vol. viii, pp. 23—60. [This paper contains references to most of Jennings's previous papers.]

v. Lendenfeld, R.—'83. 'Zeitschr. f. wiss. Zoologie,' Bd. xxxviii, pp. 355—371.

Loeb, J.—'91. 'Untersuchungen zur Physiologischen Morphologie der Thiere,' "I. Ueber Heteromorphose," Wirzburg, 1891.

Marshall, W.—'82. 'Zeitschr. f. wiss. Zoologie,' Bd. xxxvii, pp. 664—702.

Pearl, R.—'01—'03. 'Quart. Journ. Micr. Sci.'

Rösel v. Rosenhof.—1755. 'Insektenbelustigungen,' Theil iii, Nürnberg, 1755.

Schneider, C. K.—'90. 'Archiv f. mikr. Anatomie,' Bd. xxxv, pp. 321—379.

Schulze, F. E.—'71. 'Über den Ban und die Entwickelung von Cordylophora lacustris,' Leipzig, Englemann.

Scourfield, D. J.—'01. 'Journ. Queckett Microscopical Club' (Ser. 2), vol. viii, pp. 137—142.

Trembley, A.—1744. 'Mémoires pour servir à l'Histoire d'un genre de Polypes d'eau douce à Bras en form de cornes,' Paris, 1744. [There are two editions of this work, one published at Leyden. I had only the Paris edition at my disposal.]

Wilson, E. B.—'91. 'Am. Naturalist,' vol. xxv, pp. 413—433.

Zoja, R.—'90. 'Alcune Ricerche Morfologiche e Fisiologiche sull' Hydra,' Pavia, 1890. [Abstract in 'Archives Italiennes de Biologie,' tome xv, pp. 125—128.]

Zykoff, W.—'98. 'Biologisches Centralblatt,' Bd. xviii, pp. 270—272.

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